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DEVELOPMENT AND VALIDATION OF A RP - HPLC METHOD FOR THE SIMULTANEOUS DETERMINATION OF TRANEXAMIC ACID AND MEFENAMIC ACID IN PURE AND PHARMACEUTICAL DOSAGE FORM

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Abstract:

A simple, Accurate, precise method was developed for the simultaneous estimation of the Tranexamic acid and Mefenamic acid in bulk and tablet dosage form. The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. Analysis was carried out at 282 nm with column Inertsil C18 (4.6mm ×250mm, 5µm particle size), dimensions at 35^{0}C temperature. The optimized mobile phase consists of Phosphate Buffer (pH-4.8): Methanol (55:45% v/v). Flow rate was maintained at 1 ml/min. Run time was selected to be 6 min because analyze gave peak around 1.688, 3.282 ±0.02min respectively and also to reduce the total run time. The percent recovery was found to be 98.0-102% was linear and precise over the same range. Both system and method precision was found to be accurate and well within range. The analytical method was found linearity over the range 100-500mg/ml of Tranexamic acid and 30-70mg/ml of Mefenamic acid of the target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

Keywords: *Tranexamic acid, Mefenamic acid, RP-HPLC, Simultaneous estimation.*

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INTRODUCTION:

Tranexamic acid is an antifibrinolytic used to reduce or prevent hemorrhagic episodes, especially in the context of hyperfibrinolytic disorders. Tranexamic acid competitively and reversibly inhibits the activation of plasminogen via binding at several distinct sites, including four or five low-affinity sites and one high-affinity site, the latter of which is involved in its binding to fibrin. The binding of plasminogen to fibrin induces fibrinolysis - by occupying the necessary binding sites tranexamic acid prevents this dissolution of fibrin, thereby stabilizing the clot and preventing hemorrhage IUPAC name is (1r,4r)-4-(aminomethyl) cyclohexane-1-carboxylic acid. Molecular Formula is $C_8H_{15}NO_2$. Molecular weight is 157.2.

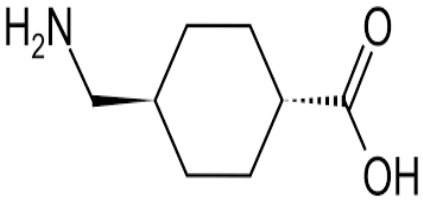

Figure 1: Structure of Tranexamic acid

Mefenamic acid is an NSAID used to treat mild to moderate pain for no more than a week, and primary dysmenorrhea. Mefenamic acid binds the prostaglandin synthetase receptors COX-1 and COX-2, inhibiting the action of prostaglandin synthetase. As these receptors have a role as a major mediator of inflammation and/or a role for prostanoid signaling in activity-dependent plasticity, the symptoms of pain are temporarily reduced. IUPAC name is 2-[(2,3-dimethylphenyl) amino] benzoic acid. Molecular Formula is $C_{15}H_{15}NO_2$. Molecular weight is 241.8.

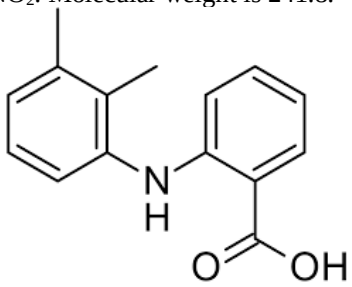

Figure 2: Structure of Mefenamic acid

The literature survey revealed that There are really few approaches reported in the literary works for evaluation of Tranexamic acid and Mefenamic acid alone or in combination with various other drugs in the pure form as well as drugs formulations by different methods [7-31]. In view of the demand for an appropriate, cost-effective RP-HPLC method for routine analysis of Tranexamic acid and Mefenamic acid synchronized evaluation of in pharmaceutical dose type. Attempts were made to establish easy, precise, accurate as well as cost-efficient logical method for the estimate of Tranexamic acid and Mefenamic acid. The recommended approach will be validated according to ICH guidelines. The objective of the recommended work is to establish a brand-new, simple, delicate, exact and economical logical method as well as recognition for the Synchronized evaluation of Tranexamic acid and Mefenamic acid in pharmaceutical dose kind by utilizing RP-HPLC. To verify the established method based on ICH standards for the desired analytical application.

MATERIALS AND METHODS:

Chemicals and Reagents:

Tranexamic acid and Mefenamic acid were Purchased from Honour Lab. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment and Chromatographic Conditions:

The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. Analysis was carried out at 282 nm with column Inertsil C18 (4.6mm ×250mm, 5µm particle size), dimensions at 35^0C temperature. The optimized mobile phase consists of Phosphate Buffer (pH-4.8): Methanol (55:45% v/v). Flow rate was maintained at 1 ml/min.

Preparation of solutions:

Preparation of mobile phase:

Accurately measured 500 ml (50%) of HPLC Methanol and 350 ml of Acetonitrile (35%) and 150 ml of Water (15%) were mixed and degassed in a digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filter.

Diluent Preparation:

Accurately measured 450 ml (45%) of HPLC Methanol and 550 ml of Phosphate Buffer (55%) were mixed and degassed in a digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filter.

Assay:

Preparation of the Tranexamic acid and Mefenamic acid standard solution:

Preparation of standard solution: (Tranexamic acid):

Accurately weigh and transfer 50 mg of Tranexamic acid , working standard into a 10ml of clean dry volumetric flasks add about 7ml of diluent and sonicate to dissolve and removal of air completely and make volume up to the mark with the diluent.

Preparation of standard solution: (Mefenamic acid):
Accurately weigh and transfer 25 mg of Mefenamic acid working standard into a 10ml of clean dry volumetric flasks add about 7ml of diluent and sonicate to dissolve and removal of air completely and make volume up to the mark with the diluent.

Further pipette 3ml of Tranexamic acid , 0.5ml of Mefenamic acid from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:
Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Preparation of Sample Solution:
Take average weight of Tablet and crush in a mortar by using pestle and weight 50 mg and 25 mg of equivalent weight of Tranexamic acid, Mefenamic acid sample into a 10ml clean dry volumetric flask and add about 7ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.
Procedure:
Further pipette 1.2ml of Tranexamic acid , Mefenamic acid from above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

RESULTS AND DISCUSSION:

METHOD:

The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters:
To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 20 µL of standard into Inertsil C18 (4.6mm ×250mm, 5µm particle size), the mobile phase of composition Phosphate Buffer (pH-4.8): Methanol (55:45% v/v) was allowed to flow through the column at a flow rate of 1.0 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

Table 1: System suitability parameters

S. NO	Parameter	Tranexamic acid	Mefenamic acid
1.	Retention Time (min)	1.688	3.282
2.	Theoretical Plates	7586	6235
3.	Tailing factor	1.69	1.58
4.	Area	1658768	426589
5.	Resolution	10.89	

Assay of pharmaceutical formulation:
The proposed validated method was successfully applied to determine Tranexamic acid and Mefenamic acid in their tablet dosage form. The result obtained for was comparable with the corresponding labeled amounts and they were shown in Table-2.

Table 2: Assay results for Tranexamic acid and Mefenamic acid

	Label Claim (mg)	% Assay
Tranexamic acid	25	99.86
Mefenamic acid	50	99.86

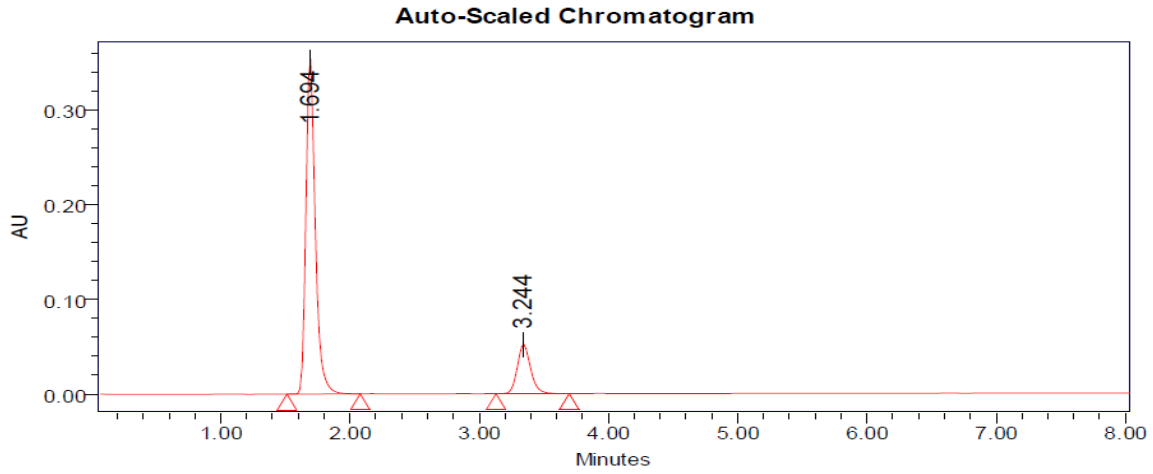

Figure 3: Standard chromatogram

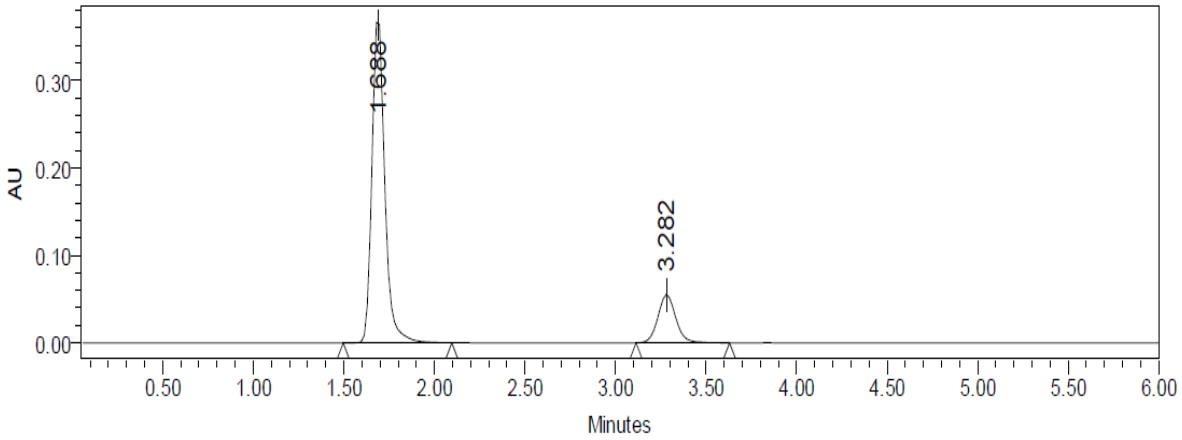

Figure 4: Sample chromatogram

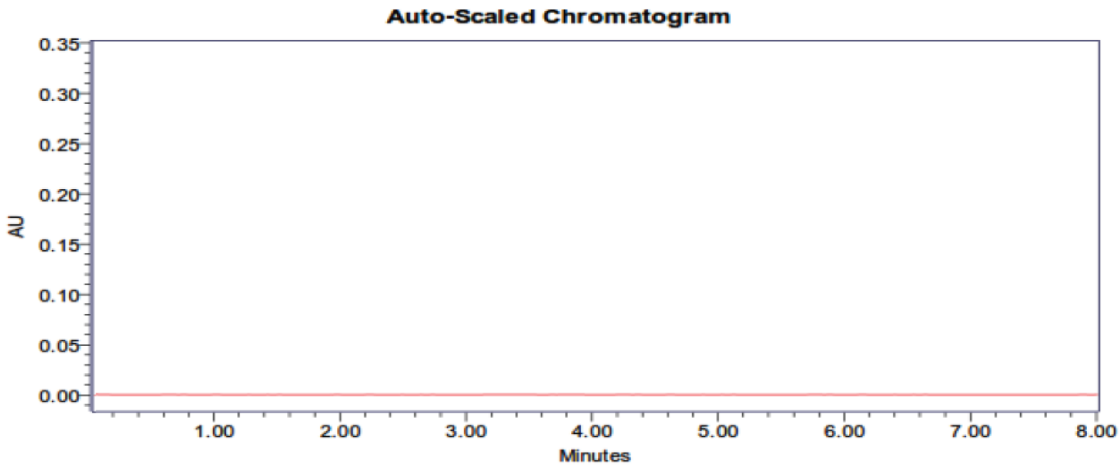

Figure 5: Blank chromatogram

Validation of Analytical method:

Linearity: The linearity study was performed for the concentration of 100 ppm to 500 ppm and 30 ppm to 70 ppm level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The results are shown in table 3,4.

Table 3: Linearity results of Tranexamic acid

S. No	Concentration Level (%)	Concentration µg/ml	Average Peak Area
1.	I	100	585985
2.	II	200	1182468
3.	III	300	1768785
4.	IV	400	2326852
5.	V	500	2856874
Correlation coefficient			0.999

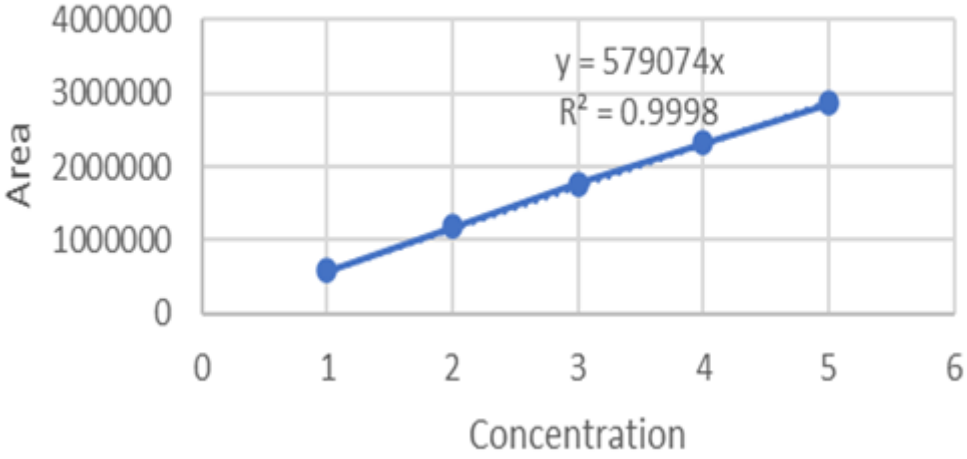

Figure 6: Linearity graph for Tranexamic acid

Table 4: Linearity results of Mefenamic acid

S. No	Concentration Level (%)	Concentration µg/ml	Average Peak Area
1	I	30	268764
2	II	40	356958
3	III	50	445631
4	IV	60	535186
5	V	70	624698
Correlation coefficient			0.999

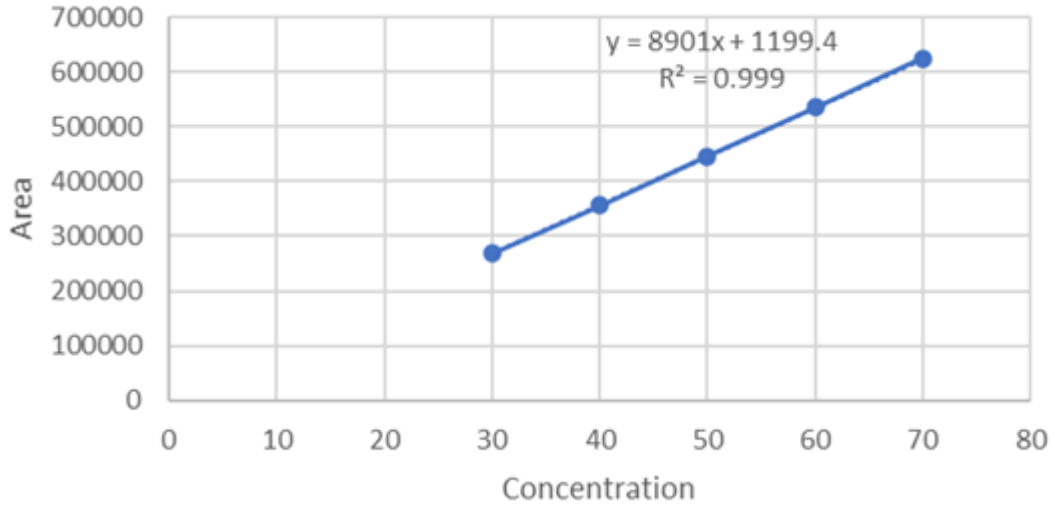

Figure 6: Linearity graph for Mefenamic acid

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 50%, 100%, 150% and 50%, 100%, 150% Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Tranexamic acid and Mefenamic acid and calculate the individual recovery and mean recovery values. The results are shown in table 5,6.

Table 5: Showing accuracy results for Tranexamic acid

%Concentration (at specification Level)	Average Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	879537	150	150.048	100.032	100.112%
100%	1743252	300	300.521	100.172	
150%	2609693	450	450.598	100.132	

Table 6: Showing accuracy results for Mefenamic acid

%Concentration (at specification Level)	Average Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	224271	25	25.114	100.456%	100.16%
100%	445748.3	50	49.952	99.904%	
150%	670006.3	75	75.101	100.134%	

Precision Studies:

precision was calculated from Coefficient of variance for five replicate injections of the standard. The standard solution was injected for five times and measured the area for all five Injections in HPLC. The %RSD for the area of five replicate injections was found. The results are shown in table 7.

Table 7: Precision results for Tranexamic acid and Mefenamic acid

S. No	Sample Area 1	Sample Area 2
1	1658254	426598
2	1658952	426589
3	1654857	426985
4	1659854	426587
5	1653298	426515
Mean	**1657043**	**426654.8**
Std.dev	**2820.29**	**187.5692**
%RSD	**0.1702**	**0.043963**

Ruggedness:

To evaluate the intermediate precision of the method, Precision was performed on different day. The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in table 8.

Table 8: Ruggedness results of Tranexamic acid and Mefenamic acid

S. No	Sample Area 1	Sample Area 2
1	1665985	436598
2	1662598	436855
3	1668484	436598
4	1664598	436587
5	1663579	436741
6	1664587	432659
Mean	**1664972**	**436006.3**
Std. Dev.	**2060.327**	**1643.285**
% RSD	**0.123745**	**0.376895**

Robustness:

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The flow rate was varied at 0.8 ml/min to 1.2 ml/min. The results are shown in table 9,10,11,12.

Table 9: Flow variation results for Tranexamic acid

Flow Rate (ml/min)		System suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
Less Flow rate	0.8	7365	1.62	1.868
Actual Flow rate	1	7586	1.69	1.688
More Flow rate	1.2	7254	1.61	1.544

Table 10: Flow variation results for Mefenamic acid

Flow Rate (ml/min)		System suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
Less Flow rate	0.8	6284	1.51	3.621
Actual Flow rate	1	6235	1.58	3.282
More Flow rate	1.2	6168	1.56	2.998

Table 11: Change in wavelength for Tranexamic acid

Organic phase		System suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
Less organic phase	50:50	7269	1.61	1.868
Actual organic phase	55:45	7586	1.69	1.688
More organic phase	60:40	7496	1.64	1.675

Table 12: Change in wavelength for Mefenamic acid

Organic phase		System suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
Less organic phase	50:50	6182	1.54	3.621
Actual organic phase	55:45	6235	1.58	3.282
More organic phase	60:40	6322	1.56	2.302

LOD and LOQ:

The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The results are shown in table 13.

LOD = 3.3σ/S and

LOQ = 10 σ/S, where

σ= Standard deviation of y intercept of regression line,

S = Slope of the calibration curve

Table 13: LOD, LOQ of Tranexamic acid and Mefenamic acid

Drug	LOD	LOQ
Tranexamic acid	2.1	1.28
Mefenamic acid	6.3	3.84

CONCLUSION:

The Developed HPLC method was validated and it was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Tranexamic acid and Mefenamic acid in its bulk and tablet dosage form. Hence, this method can easily and conveniently adopt for routine quality control analysis of Mefenamic acid and Tranexamic acid in its bulk and tablet dosage form.

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