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HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF TRANEXAMIC ACID AND ETHAMSYLATE PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Tranexamic acid and Ethamsylate in Tablet dosage form. Chromatogram was run through Kromasil 250 x 4.6 mm, 5µ. Mobile phase containing Buffer and Acetonitrile in the ratio of 55:45A was pumped through column at a flow rate of 1ml/min. Buffer used in this method was 0.1% OPA buffer with pH 4.8 adjusted by Triethylamine and Temperature was maintained at 30°C. Optimized wavelength for Tranexamic acid and Ethamsylate was 230nm. Retention time of Tranexamic acid and Ethamsylate were found to be 2.527min and 3.142 min. %RSD of the Tranexamic acid and Ethamsylate were and found to be 0.9 and 0.1 respectively. %assay was obtained as 100.54% and 99.98% for Tranexamic acid and Ethamsylate respectively. LOD, LOQ values are obtained from regression equations of Tranexamic acid and Ethamsylate were 0.02ppm, 0.08ppm and 0.07ppm, 0.24ppm respectively. Regression equation of Tranexamic acid & Ethamsylate is y = 18625x + 4734and y = 18614x + 5549.Retention times are decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

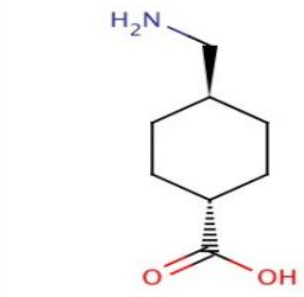
KEYWORDS: Tranexamic acid, Ethamsylate, RP-HPLC.

Drug Profiles

1. Tranexamic Acid

Description: Antifibrinolytic haemostatic used in severe hemorrhage.

Structure



Appearance: Crystalline powder
Molecula Weight: 157.21
Molecularformula: $C_8H_{15}NO_2$
Iupac: (1r,4r)-4-(aminomethyl)cyclohexane-1-carboxylic acid.

Indication: For use in patients with hemophilia for short term use (two to eight days) to reduce or prevent hemorrhage and reduce the need for replacement therapy during and following tooth extraction. It can also be used for excessive bleeding in menstruation, surgery, or trauma cases.

Pharmaco Dynamics: Tranexamic acid is an antifibrinolytic that competitively inhibits the activation of plasminogen to plasmin. Tranexamic acid is a competitive inhibitor of plasminogen activation, and at much higher concentrations, a noncompetitive inhibitor of plasmin, i.e., actions similar to aminocaproic acid. Tranexamic acid is about 10 times more potent in vitro than aminocaproic acid. Tranexamic acid binds more strongly than aminocaproic acid to both the strong and weak receptor sites of the plasminogen molecule in a ratio corresponding to the difference in potency between the compounds. Tranexamic acid in a concentration of 1 mg per mL does not aggregate platelets in vitro. In patients with hereditary angioedema, inhibition of the formation and activity of plasmin by tranexamic acid may prevent attacks of angioedema by decreasing

plasmin-induced activation of the first complement protein (C1).

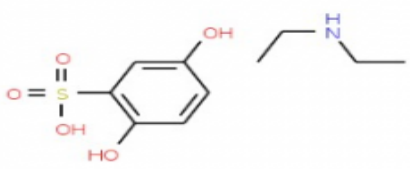
Mechanism of Action: Tranexamic acid competitively inhibits activation of plasminogen (via binding to the kringle domain), thereby reducing conversion of plasminogen to plasmin (fibrinolysin), an enzyme that degrades fibrin clots, fibrinogen, and other plasma proteins, including the procoagulant factors V and VIII. Tranexamic acid also directly inhibits plasmin activity, but higher doses are required than are needed to reduce plasmin formation.

Absorption: Absorption of tranexamic acid after oral administration in humans represents approximately 30 to 50% of the ingested dose and bioavailability is not affected by food intake.

2. Ethamsylate

Description: Ethamsylate Is An Organic Acid Widely Distributed In Animal Tissues. It is a Major Constituent of Bile and can be found in the Large Intestine,

Structure



Appearance: Solid
Molecular Weight: 263.31
Molecular Formula: $C_6H_6OS_5 \bullet C_4H_{11}N$

Indication: Prophylaxis and control of haemorrhages from small blood vessels, neonatal intraventricular haemorrhage capillary bleeding of different etiology, including: menorrhagia and metrorrhagia without organic pathology, after trans-urethral resection of the prostate, hematemesis, melena, hematuria, epistaxis; secondary bleeding due to thrombocytopenia or thrombocytopathia, hypocoagulation, prevention of periventricular hemorrhages in prematurely born children

Mechanism of Action: Catalyzes the conversion of Ethamsylate and alpha ketoglutarate to sulfite, aminoacetaldehyde and succinate. Required for the utilization of Ethamsylate (2-aminoethanesulfonic acid) as an alternative sulfur source. Pentane-sulfonic acid, 3-(N-morpholino)propanesulfonic acid and 1,3-dioxo-2-isoindolineethanesulfonic acid are also substrates for this enzyme.

Category: Catalytic activity, oxidoreductase activity.

OBJECTIVE AND PLAN OF STUDY

- To develop a new HPLC method for simultaneous estimation of Tranexamic acid and Ethamsylate to develop a validated method according to ICH guidelines.
- To apply validated method for the estimation of Tranexamic acid and Ethamsylate in pharmaceutical formulation.

MATERIALS AND METHODS

Materials

Tranexamic acid and Ethamsylate, Combination Tranexamic acid and Ethamsylate tablets, distilled water, acetonitrile, phosphate buffer, ammonium acetate buffer, glacial acitic acid, methanol, potassium dihydrogen phosphate buffer, tetra hydrofuran, tri ethyl amine, ortho-phosphoric acid etc.

Instrument

HPLC instrument used was of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Software used is Empower 2. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was be used for measuring absorbance for Tranexamic acid and Ethamsylate solutions.

Methods

Preparation of buffer

Buffer: (0.1 %OPA)

1 ml of con. OPA is dissolved in 1000 ml volumetric flask diluted with distilled water up to the mark. pH adjusted to 2.8 by using Triethylamine.

Standard Preparation

Accurately Weighed and transferred 20mg&10mg of Tranexamic acid and Ethamsylate working Standards into a 10ml and 10ml clean dry volumetric flask respectively, add 5ml and 5ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents. From the above stock solutions, 1ml was pipette out in to a 10ml volumetric flask and then make up to the final volume with diluent.

Sample Preparation

5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 1 tablet was transferred into a 250 ml volumetric flask, 150ml of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pipette out into a 10 ml volumetric flask and made up to 10ml with diluent.

Linearity: Linearity solutions are prepared such that 0.25ml, 0.5ml, 0.75ml, 1ml, 1.25ml, 1.5ml from the Stock solutions Tranexamic acid and Ethamsylate are taken in to 6 different volumetric flasks and diluted to 10ml with diluents to get 50ppm, 100ppm, 150ppm, 200ppm, 250 ppm, 30 ppm of Tranexamic acid and 25 ppm, 50ppm, 75 ppm, 100ppm, 125ppm, 150 ppm of Ethamsylate

Accuracy

Standard Preparation

Accurately Weighed and transferred 20mg&10mg of Tranexamic acid and Ethamsylate working Standards into a 10ml and 10ml clean dry volumetric flask respectively, add 5ml and 5ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents.

Preparation of 50% Spiked Solution: weight equivalent to 500mg of tablet powder was transferred into a 250 ml volumetric flask, 50ml of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered. 1ml from each standard stock solution was pipette out and taken into a 10ml volumetric flask to that 1ml of filtered Accuracy 100% Sample stock solution was spiked and made up with diluents.

Preparation of 100% Spiked Solution: weight equivalent to 1000mg of tablet powder was transferred into a 250 ml volumetric flask, 50ml of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered. 1ml from each standard stock solution was pipette out and taken into a 10ml volumetric flask to that 1ml of filtered Accuracy 100% Sample stock solution was spiked and made up with diluents.

Preparation of 150% Spiked Solution: weight equivalent to 1500 mg of tablet powder was transferred into a 250 ml volumetric flask, 750ml of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered. 1ml from each standard stock solution was pipette out and taken into a 10ml volumetric flask to that 1ml of filtered Accuracy 100% Sample stock solution was spiked and made up with diluents.

Method Development

Method Development: Many trials were done by changing columns and Mobile phases and were reported below.

Trial: 1

Column Used : Kromasil 250 x 4.6 mm, 5µ.
Mobile phase : water: methanol (50:50)
Flow rate : 1ml/min
Wavelength : 230nm
Temperature : 30°C
Injection Volume : 10µl

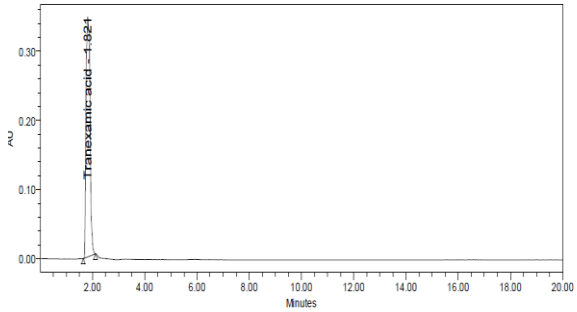


Fig 1: Trial chromatogram.

Observation: Tranexamic acid peak was eluted but not eluted Ethamsylate peak so further trail was carried out.

Trial: 2

Column Used : Kromasil 250 x 4.6 mm, 5µ.
Mobile phase : Buffer: Acetonitrile (30:70A)
Buffer : 0.1% Ortho phosphoric acid
Flow rate : 1ml/min
Wavelength : 230nm
Temperature : 30°C
Injection Volume : 10µl

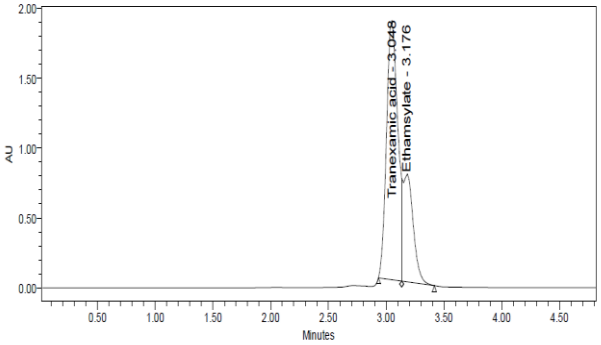


Fig 2: Trial chromatogram.

Observation: Tranexamic acid and Ethamsylate both peaks eluted but. Peak shape was not good and resolution less So further trial is carried out.

Optimized Method: Drugs were eluted with good retention time, resolution; all the system suitable parameters like Plate count and Tailing factor were within the limits.

Column Used : Kromasil 250 x 4.6 mm, 5µ.
Buffer used : OPA
Mobile phase : Buffer: Acetonitrile (55:45A)
Flow rate : 1ml/min
Diluent : Water and Acetonitrile (50:50).
Wavelength : 230
Temperature : 30°C
Injection Volume : 10µl

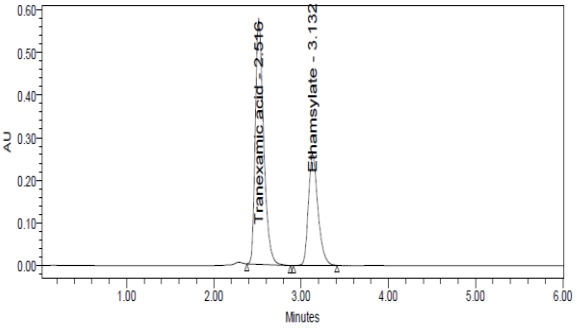


Fig 3: Optimized chromatogram of Tranexamic acid and Ethamsylate.

Observation: peak shape and retention time is good

RESULTS AND DISCUSSIONS

1. **System suitability:** All the system suitability parameters are within range and satisfactory as per ICH guidelines.

System suitability studies of Tranexamic acid and Ethamsylate method.

Property	Tranexamic acid	Ethamsylate
Retention time (t_R)	2.527min	3.142min
Theoretical plates (N)	3341 ± 63.48	4096 ± 63.48
Tailing factor (T)	1.37 ± 0.117	1.29± 0.117

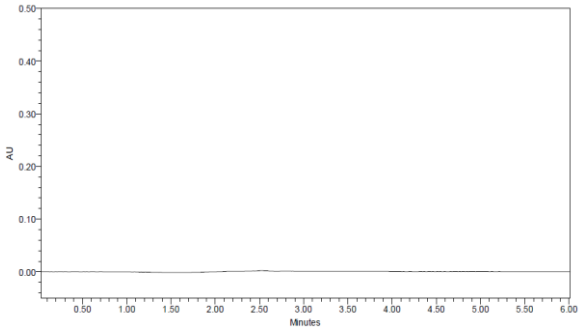


Fig 4: Chromatogram of blank.

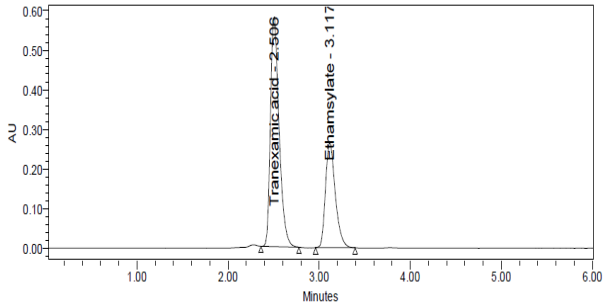


Fig 5: Typical chromatogram of Tranexamic acid and Ethamsylate.

2. **Linearity:** Six Linear concentrations of Tranexamic acid (50-300ppm) and Ethamsylate (25-150ppm) are prepared and Injected. Regression equation of the Tranexamic acid and Ethamsylate are found to be, y = 18625.x + 4734, and y = 18614.x + 5549. And regression co-efficient was 0.999.

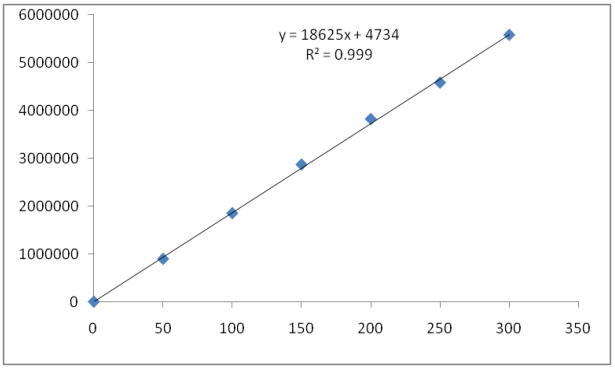


Fig 6: Calibration curve of Tranexamic acid.

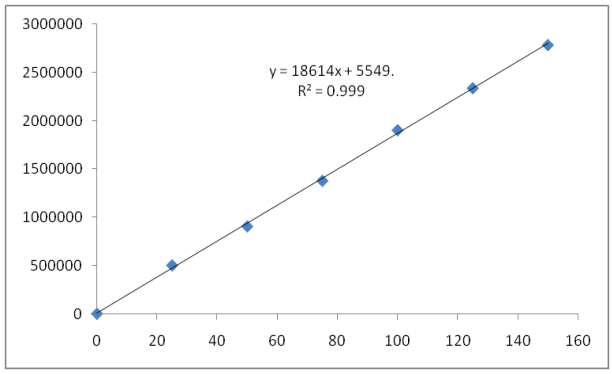


Fig 7: Calibration curve of Ethamsylate.

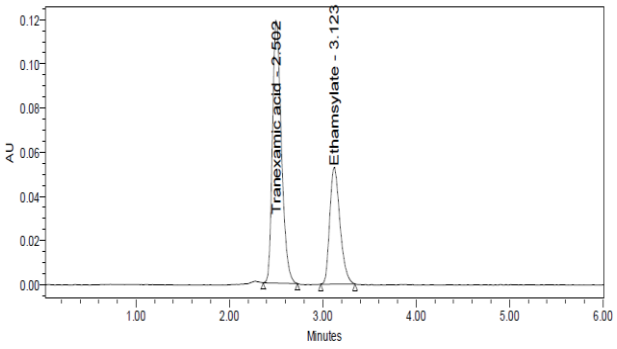


Fig 8: Linearity 25% Chromatogram of Tranexamic acid and Ethamsylate.

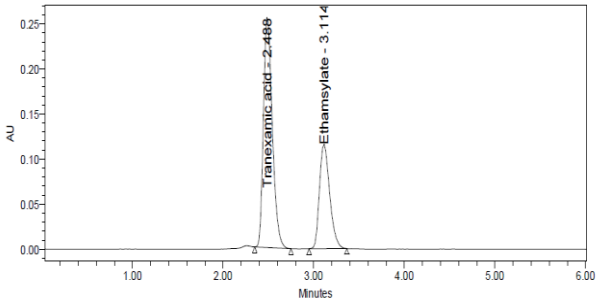


Fig 9: Linearity 50% Chromatogram of Tranexamic acid and Ethamsylate.

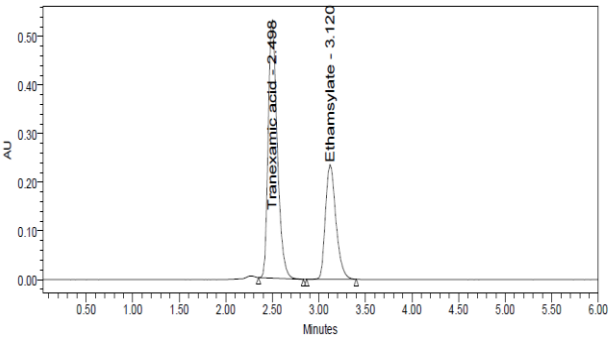


Fig 10: Linearity 75% Chromatogram of Tranexamic acid and Ethamsylate.

3. Precision

Intraday precision (Repeatability): Intraday Precision was performed and % RSD for Tranexamic acid and Ethamsylate were found to be 0.9% and 0.1% respectively.

Repeatability results for Tranexamic acid and Ethamsylate.

S.No.	Tranexamic acid	Ethamsylate
1	3865035	1990117
2	3947651	1987499
3	3865633	1987087
4	3867298	1992210
5	3856195	1989632
6	3859513	1984121
Mean	3876888	1988444
Std. Dev.	34918.8	2821.9
%RSD	0.9	0.1

*Average of six determinations

4. **Accuracy:** Three concentrations 50%, 100%, 150%, were injected in a triplicate manner and amount Recovered and % Recovery were displayed in Table 6.5.

Sample	Concentration (%) (µg/ml)	Recovery (%)	% RSD
Tranexamic acid	100	99.65	0.26
	200	100.55	1.20
	300	100.25	0.30
Ethamsylate	50	99.78	1.19
	100	99.50	0.52
	150	100.21	1.42

5. **LOD:** Limit of detection was calculated by std deviation method Tranexamic acid and Ethamsylate and LOD for Tranexamic acid and Ethamsylate were found to be 0.02and 0.08respectively.

6. **LOQ:** Limit of Quantification was calculated by std deviation method Tranexamic acid and Ethamsylate and LOQ for Tranexamic acid and Ethamsylate were found to be 0.07and 0.24 respectively.

7. **Robustness:** Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.

Assay: Standard preparations are made from the API and Sample Preparations are from Formulation. Both sample and standards are injected six homogeneous samples. Drug in the formulation was estimated by taking the standard as the reference. The Average %Assay was calculated and found to be 100.54% and 99.98% for Tranexamic acid and Ethamsylate respectively.

Assay of Tablet

S. No.	Tranexamic acid %Assay	Ethamsylate %Assay
1	100.23	100.06
2	102.37	99.93
3	100.24	99.91
4	100.29	100.17
5	100.00	100.04
6	100.09	99.76
AVG	100.54	99.98
STDEV	0.9055	0.1419
%RSD	0.9	0.14

SUMMARY AND CONCLUSION

Summary Table

Parameters	Tranexamic acid	Ethamsylate
Calibration range (mcg / ml)	50-300ppm	25-150ppm
Optimized wavelength	230nm	230nm
Retention time	2.527min	3.142 min
Regression equation (Y*)	y = 18625.x + 1734	y = 18614.x + 5549
Correlation coefficient(r^2)	0.999	0.999
Precision (% RSD*)	0.9	0.1
% Assay	100.54	99.98
Limit of Detection (mcg / ml)	0.02ppm	0.08ppm
Limit of Quantization (mcg / ml)	0.07ppm	0.24ppm

CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Tranexamic acid and Ethamsylate in Tablet dosage form. Retention time of Tranexamic acid and Ethamsylate were found to be 2.527min and 3.142 min. %RSD of the Tranexamic acid and Ethamsylate were and found to be 0.9 and 0.1 respectively. %assay was obtained as 100.54% and 99.98% for Tranexamic acid and Ethamsylate respectively. LOD, LOQ values are obtained from regression equations of Tranexamic acid and Ethamsylate were 0.02ppm, 0.08ppm and 0.07ppm, 0.24ppm respectively. Regression equation of Tranexamic acid & Ethamsylate is y = 18625.x + 4734and y = 18614.x + 5549. Retention times are decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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