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R E S E A R C H A R T I C L E

Analytical Method Development and Validation for Tranexamic Acid and Ethamsylate in Combine Dosage Form by RP-HPLC

[1]V. Bhavani*, [2]Priyanka K

Department of Pharmaceutical Analysis, *Smt. Sarojini Ramulamma college of Pharmacy, Sheshadri Nagar, Mahabubnagar, Telangana, India.*

A B S T R A C T

A new method was established for simultaneous estimation of Tranexamic acid and Ethamsylate by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Tranexamic acid and Ethamsylate by using Inertsil C18 5µm (4.6*250mm) column, flow rate was 1ml/min, mobile phase ratio was Phosphate buffer (0.05M) pH 3: MEOH (30:70%v/v) (pH was adjusted with orthophosphoric acid), detection wave length was 240nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, PDA Detector 996, Empower-software version-2. The % purity of Tranexamic acid and Ethamsylate was found to be 98.95% and 100.25% respectively. The system suitability parameters for Tranexamic acid and Ethamsylate such as tailing factor and theoretical plates were found to be 1.2, 4683.4 and 1.3, 6490.3 the resolution was found to be 6.0. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study for Tranexamic acid and Ethamsylate was found in concentration range of 50µg-450µg and correlation coefficient (r2) was found to be 0.998 and 0.997. % mean recovery was found to be 100.53 and 99.88. %RSD for repeatability was 0.5 and 0.3 and %RSD for intermediate precision was 0.2 and 0.1 respectively. The precision study was precise, robust, and repeatable. LOD value was 2.8 and 0.2 and LOQ value was 10.01 and 10.3 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Tranexamic acid and Ethamsylate in API and Pharmaceutical dosage form.

Keywords: Inertsil C18, Ethamsylate and Tranexamic acid RP-HPLC

A R T I C L E I N F O

Corresponding Author
V. Bhavani
Department of Pharmaceutical Analysis,
Smt. Sarojini Ramulamma College of Pharmacy,
Mahabubnagar, Telangana, India.
MS-ID: IJMPR4024

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CONTENTS

1. Introduction

Analytical chemistry is a branch of chemistry involved in separating, identifying and determining the relative amounts of the components making up a sample of matter. It is mainly involved in the qualitative analysis or detection of compounds and quantitative analysis of the compounds. A qualitative method yields information about the identity of atomic or molecular species or functional groups in the sample. A quantitative method, in contrast provides numerical information as to the relative amount of one or more of these components.

High Performance Liquid Chromatography (HPLC)

A variety of methods are available for analyzing pharmaceutical compounds. High Performance/Pressure Liquid Chromatography (HPLC) is one of the best methods of choice for analyzing a variety of natural and synthetic compounds. It is because it offers high performance over ambient pressure.

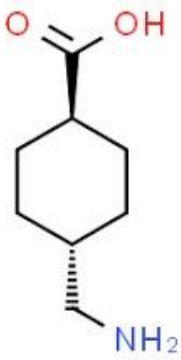

Fig.1. Tranexamic Acid

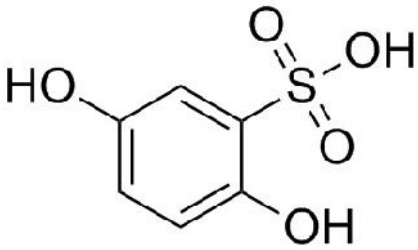

Fig.2. Ethamsylate

2. Materials and Methods

HPLC WATERS, software: Empower, 2695 separation module, PDA detector. UV/VIS spectrophotometer LABINDIA UV $3000^{+,}$ pH meter, Weighing machine, Pipettes and Burettes, Beakers. Ethamsylate and Tranexamic acid, KH_2PO_4, Water and Methanol for HPLC, Acetonitrile for HPLC, Ortho phosphoric Acid.

Chromatographic conditions (optimized method).

Trial 5:

Mobile phase : Phosphate buffer pH 3.0: Methanol (30:70%v/v)
Column : Inertsil C18 5µm (4.6*250mm)
Flow rate : 1.0 ml/min
Wavelength : 240 nm
Column temp : Ambient
Injection Volume: 10 µl
Run time: 8 min

Retention time: Tranexamic acid-2.425 and Ethamsylate-3.865

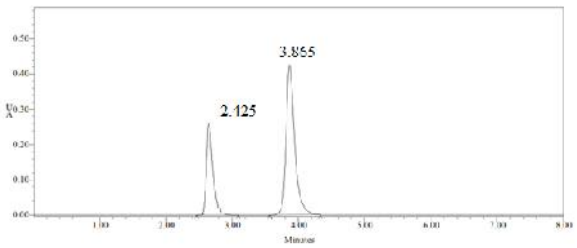

Fig.1 Chromatogram for Ethamsylate and Tranexamic acid sample Preparation

From the above chromatogram it was observed that the Ethamsylate and Tranexamic acid peaks are well separated. Both the components were eluted with good retention times & peak shapes.

Standard Solution Preparation:

Accurately weigh and transfer 10 mg of Ethamsylate and Tranexamic acid 10mg of working standard into a 10mL& 100ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 2.5ml of the above each stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation:

Accurately weigh 10 tablets crush in mortor and pestle and transfer equivalent to 10 mg of Ethamsylate and Tranexamic acid (marketed formulation) sample into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 2.5 ml of Ethamsylate and Tranexamic acid of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Method Validation

PRECISION:

Accurately weigh and transfer 10 mg of Ethamsylate and Tranexamic acid working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Intermediate Precision/Ruggedness:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions.

Accuracy: Accurately weigh and transfer 10 mg of Ethamsylate and Tranexamic acid 10mg of working standard into a 10mLclean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Linearity:

Accurately weigh 10 tablets crush in mortor and pestle and transfer equivalent to 10 mg of Ethamsylate and Tranexamic acid (marketed formulation) sample into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Limit of Detection: Accurately weigh and transfer 10 mg of Ethamsylate working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Limit of Detection: Accurately weigh and transfer 10mg of Tranexamic acid working standard into a 10ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Limit of Quantification

Accurately weigh and transfer 10 mg of Ethamsylate working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Limit of Quantification

Accurately weigh and transfer 10mg of Tranexamic acid working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

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.

3. Results and discussion

Table 1: Results of system suitability parameters for Ethamsylate and Tranexamic acid

S.No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	**Tranexamic acid**	2.5	124505	154566		1.2	4683.4
2	**Ethamsylate**	3.9	1308495	213642	6 0	1.3	6490.3

Table 2: Results of method precession for Tranexamic acid

Injection	Area
Injection-1	123149
Injection-2	125766
Injection-3	124272
Injection-4	124690
Injection-5	124952
Average	123162.7
Standard Deviation	726.6
%RSD	0.5

Table 3: Results of method precession for Ethamsylate

Injection	Area
Injection-1	1102727
Injection-2	1102947
Injection-3	1103236
Injection-4	1103977
Injection-5	1109759
Average	1104529.8
Standard Deviation	2561.2
%RSD	0.3

TABLE 4: Results of Intermediate precision for Tranexamic acid

Injection	Area
Injection-1	122488
Injection-2	121627
Injection-3	122632
Injection-4	122706
Injection-5	122965
Average	122685.8
Standard Deviation	184.8
%RSD	0.2

TABLE 5: Results of Intermediate precision for Ethamsylate

Injection	Area
Injection-1	1108147
Injection-2	1104523
Injection-3	1105836
Injection-4	1105478
Injection-5	1108767
Average	1205078.3
Standard Deviation	2061.7
%RSD	0.1

Table-6 accuracy (recovery) data for Tranexamic acid

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	65800	5.3	5.34	100.8%	100.53%
100%	124353	10	10.10	100.01%	
150%	177940	15.0	14.45	99.68%	

Table-7 accuracy (recovery) data for Ethamsylate

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	656659.5	5.0	5.036	100.7%	99.88%
100%	1304258	10.0	10.003	100.0%	
150%	1854608	15.0	14.224	98.780%	

Table-8 Area of different concentration of Tranexamic acid

S.No	Linearity Level	Concentration	Area
1	I	50ppm	20010
2	II	150ppm	71701
3	III	250ppm	113802
4	IV	350ppm	159731
5	V	450ppm	199732
Correlation Coefficient			0.997

Table-9 Area of different concentration of Ethamsylate

S.No	Linearity Level	Concentration	Area
1	I	50ppm	208934
2	II	150ppm	704781
3	III	250ppm	1103873
4	IV	350ppm	1523458
5	V	450ppm	1906084
Correlation Coefficient			0.996

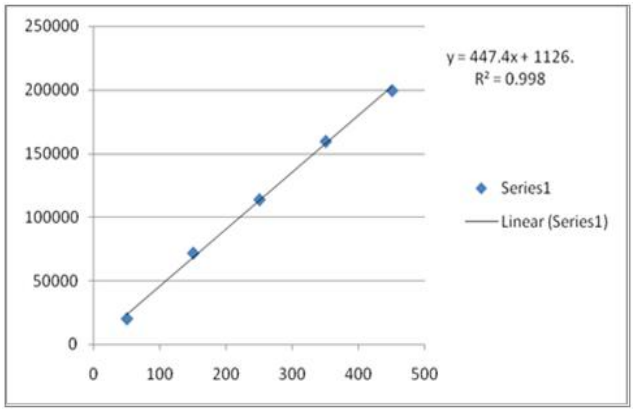

Fig.3. calibration graph for Tranexamic acid

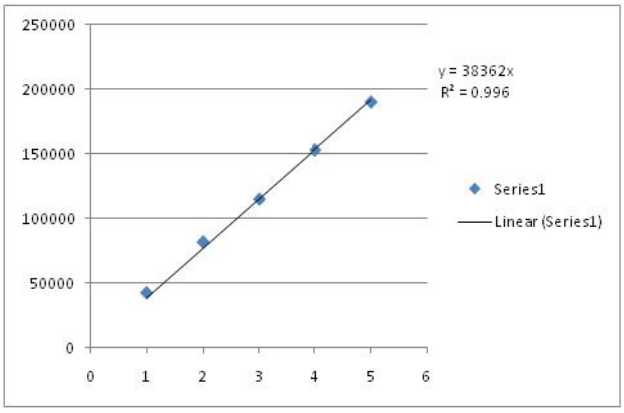

Fig. 4.Calibration graph for Ethamsylate

Table-10 Analytical performance parameters of Tranexamic acid and Etahmsylate

Parameters	Tranexamic acid	Ethamsylate
Slope (m)	12528	67574
Intercept (c)	50248	53593
Correlation coefficient (R^2)	0.997	0.999

Table-11 Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Tranexamic acid	53	153	2.8
Ethamsylate	53	153	0.2

Table no-12 Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Tranexamic acid	51	423	10.01
Ethamsylate	51	423	10.3

Table-13 Flow Rate (ml/min) data for Tranexamic acid

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	5339.9	1.4
2	1.0	4673.4	1.3
3	1.2	5216.0	1.4

Table-14 flow rate (ml/min) data for Ethamsylate

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	7063.3	1.3
2	1.0	6090.3	1.2
3	1.2	6998.0	1.3

Table -15 Change in Organic Composition in the Mobile Phase for Tranexamic acid

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	4508.4	1.3
2	*Actual	4673.4	1.4
3	10% more	4318.1	1.3

Table -16 Change in Organic Composition in the Mobile Phase for Ethamsylate

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	6387.7	1.2
2	*Actual	6090.3	1.2
3	10% more	6232.5	1.2

4. Conclusions

A new method was established for simultaneous estimation of Tranexamic acid and Ethamsylate by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Tranexamic acid and Ethamsylate by using Inertsil C18 5µm (4.6*250mm) column, flow rate was 1ml/min, mobile phase ratio was Phosphate buffer (0.05M) pH 3: MEOH (30:70%v/v) (pH was adjusted with orthophosphoric acid), detection wave length was 240nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, PDA Detector 996, Empower-software version-2. The % purity of Tranexamic acid and Ethamsylate was found to be 98.95% and 100.25% respectively. The system suitability parameters for Tranexamic acid and Ethamsylate such as tailing factor and theoretical plates were found to be 1.2, 4683.4 and 1.3, 6490.3 the resolution was found to be 6.0. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study for Tranexamic acid and Ethamsylate was found in concentration range of 50µg-450µg and correlation coefficient (r2) was found to be 0.998 and 0.997. % mean recovery was found to be 100.53 and 99.88. %RSD for repeatability was 0.5 and 0.3 and %RSD for intermediate precision was 0.2 and 0.1 respectively. The precision study was precise, robust, and repeatable. LOD value was 2.8 and 0.2 and

LOQ value was 10.01 and 10.3 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Tranexamic acid and Ethamsylate in API and Pharmaceutical dosage form.

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