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Analytical Method Development and Validation for the Simultaneous Estimation of Tranexamic acid and Ethamsylate by RP-HPLC Method in Bulk and Pharmaceutical Dosage Form

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A B S T R A C T

The chromatographic conditions were successfully developed for the separation of Tranaxamic acid and Ethamsylate by using Thermosil C18 column (4.6×100mm) 5µ, flow rate was 1ml/min, mobile phase ratio was Methanol: Phosphate buffer P^H 3 (35:65 v/v), detection wavelength was 256 nm. The Spectroscopic method was done in solvent using methanol and the instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.466 mins and 4.337 mins. The % purity of Tranaxamic acid and Ethamysylate was found to be 99.83% and 98.89% respectively. The system suitability parameters for Tranaxamic acid and Ethamysylate such as theoretical plates and tailing factor were found to be 2095, 1.6 and 2766 and 1.2, the resolution was found to be 5.4. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Tranexamic acid and Ethamysylate was found in concentration range of 10µg-50µg and 60µg-300µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 98.54% and 100.07%, %RSD for repeatability was 0.36 and 0.46, % RSD for intermediate precision was 0.1 and 0.1 respectively. The precision study was precision, robustness and repeatabilty.LOD value was 3.052 and 3.402 and LOQ value was 9.85 and 10.12 respectively.

Keywords: Tranexamic acid, Ethamsylate, HPLC

A R T I C L E I N F O

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1. Introduction

Analytical methods:

Methods are developed for new products when no official methods are available. Alternate methods for existing (non-pharmacopoeial) products are developed to reduce the cost and time for better precision and ruggedness [1]. Trial runs are conducted, method is optimized and validated. When alternate method proposed is intended to replace the existing procedure comparative laboratory data including merit/demerits are made available [2].

Description of the Various Analytical Methods

Titrimetric and gravimetric method of analysis is suitable when the sample is present in pure form or when no interference is observed in the mixture with other materials [3]. Ultraviolet and visible spectrometric method is suitable when no Interference is observed in the mixture [4,5]. HPLC and GC methods are more advantageous than the above due to their capability in separating organic mixtures and quantitative estimations. AAS is used mainly for quantitative estimation in ppm and ppb levels of elements. Infra-red spectroscopy though mainly used for qualitative analysis can be used for quantitative estimation also. Out of all the above methods, thin layer chromatography plays a very important role in analysis due to its adaptability, flexibility, and cost and time. It can be used both for qualitative and quantitative determination. After separation spots can be scanned with the help of a scanner and quantitative measurement can be made [6].

Chromatography:

Chromatography is a technique used in analytical chemistry to separate and identify components of mixtures. The name comes from the Greek term for "color writing" because this method was originally used to separate colored samples. The advent of high-performance liquid chromatography (HPLC).in this system pressure is applied to the column, forcing the mobile phase through at much higher rate [7]. The pressure is applied using a pumping system. The action of the pump is critical, since it must not pulsate and mix up the sample being separated in the solvent, causing it to lose resolution [8]. Development of pumps has proceeded quite quickly over the last several years, and now it is possible to achieve good resolution under the conditions required for HPLC [9].

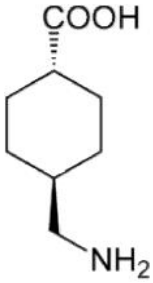

Figure 1: Tranaxamic acid

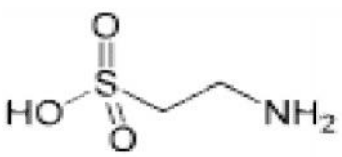

Figure 2: Ethamsylate

2. Materials and Methods

Apparatus

The instrument used for the study was Waters, Empower 2695 separation module 2996, PDA detector. Empower 2 software.

Reagents and Materials

The solvents used were Potassium dihydrogen orthophosphate, Sodium perchlorate, Perchloric acid, Methanol, Ortho phosphoric acid, Acetonitril, and Water

Selection of detection wavelength:

A solution of 10 µg/ml of Tranexamic acid and Ethamsylate were prepared in milliQ water. The resulting solutions were scanned individually on HPLC PDA detector from 200 to 400 nm and also in UV-Visible spectrophotometer. The optimal response for three of them was obtained at 256 nm. Hence the complete method was processed at the wavelength of 256nm [10].

Selection of mobile phase

Initially the mobile phase tried was methanol and water, methanol and Methanol, buffer and water in various proportions. Finally, the mobile phase was optimized to Methanol: Phosphate buffer in proportion 35:65 v/v respectively [11].

Optimization Chromatographic trials for Simultaneous Estimation of Tranexamic acid and Ethamsylate by RP-HPLC.

Optimization Chromatographic conditions

Column : Thermosil C_{18} (4.6 x 100mm, 5µm)
Mobile phase ratio: Methanol: Phosphate buffer P^H 3 (35:65 v/v)
Detection wavelength: 256 nm
Flow rate : 1.0ml/min
Injection volume : 10µl
Column temperature : Ambient
Auto sampler temperature : Ambient
Run time : 10min
Retention time : 2.972 and 3.548 mins

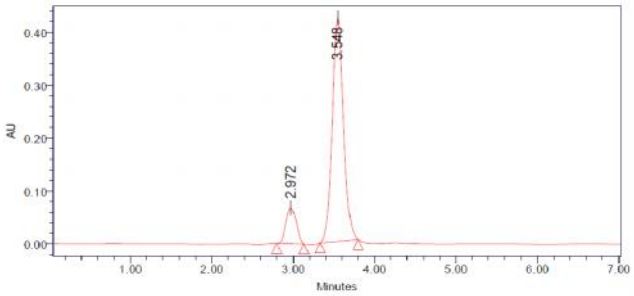

Figure 3: Optimization Chromatogram

Observation: Resolution between two analytes were good. No peak asymmetry was observed. No other impurity interference was seen. All the results were found to be with in the acceptance criteria. Hence the method was considered to be optimized.

Procedure

Preparation of phosphate buffer

About 7.0g of potassium dihydrogen orthophosphate was dissolved in 1000ml of HPLC grade water and pH 3 was

adjusted with orthophosphoric acid. It was filtered through 0.45µm nylon membrane filter and degassed with sonicator. It was used as a diluent for the preparation of sample and standard solution [12].

Preparation of mobile phase

Take 24 gm of Sodium acetate into 1000ml volumetric flask dissolved in HPLC grated water and adjust Ph up to 3 with ortho phosphoric acid. From the above prepared buffer take 350 ml (35%) and 650ml Methanol (65%) HPLC were mixed and degassed in ultrasonic water bath for 5 minutes and was filtered through 0.45 µ filter under vacuum filtration.

Tranexamic acid &Ethamsylate standard preparations

10 mg of Tranexamic acid and 10mg of Ethamsylate were accurately weighed and transferred into a 10 ml clean dry volumetric flask, about 7 ml of diluent was added, sonicated to dissolve it completely and the volume was made up to the mark with the same solvent to give a concentration of 1000 µg/ml. (Stock solution) Further 0.3 and 1.8 ml were pipetted out from the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent to give a concentration of 30 µg/ml and 180µg/ml respectively[13].

Sample solutions preparation [14]

10 Tablets of contents were weighed and triturated in glass mortar. The quantity of powder equivalent to 10 mg of active ingredient present in Tranexamic acid and Ethamsylate was transferred into a 10 ml clean dry volumetric flask, 7 ml of diluent was added to it and was shaken by mechanical stirrer and sonicated for about 30 minutes by shaking at intervals of five minutes each and was diluted up to the mark with diluent to give a concentration of 1000 µg/ml and allowed to stand until the residue settles before taking an aliquot for further dilution (stock solution). 0.3 ml of upper clear solution was transferred to a 10 ml volumetric flask and diluted with diluent up to the mark to give the respective concentrations as par with standard solution. The solution was filtered through 0.45 µm filter before injecting into HPLC system

3. Results and Discussion

Method Validation Parameters

1. Specificity

ICH defines specificity as "the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically this might include impurities, degradants, matrix, etc [15].

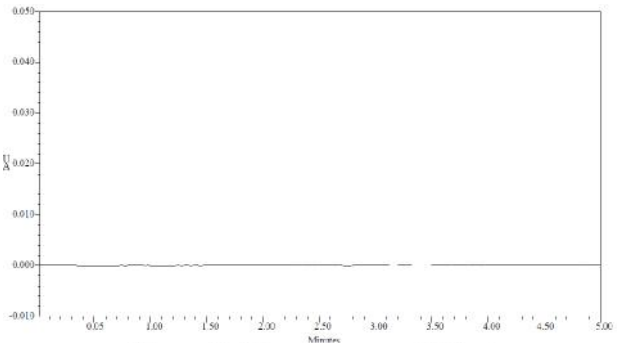

Figure 4: Chromatogram of Blank

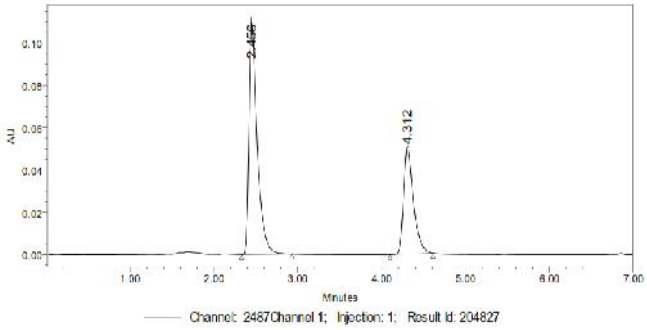

Figure 5: Chromatogram of Sample

2. Linearity

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. Serial dilutions of Tranexamic acid and Ethamsylate (10-50µg/ml and 60-300 µg/ml) were injected into the column and detected at a wavelength set at 256 nm. The calibration curve was obtained by plotting the concentration vs. peak area.

Acceptance criteria: Correlation coefficient should be not less than 0.999.

3. Range

Based on precision, linearity and accuracy data it can be concluded that the assay method is precise, linear and accurate in the range of 10µg/ml-50µg/ml and 60µg/ml to 30µg/ml of Tranexamic acid and Ethamsylate respectively.

4. Accuracy

Accuracy of the method was determined by recovery experiments. There are mainly 2types of recovery studies are there.

- Standard addition method: To the formulation, the reference standard of the respective drug of known concentration was added, analyzed by HPLC and compared with the standard drug concentration.
- Percentage method: For these assay method samples are prepared in three concentrations of 50%, 100%, and 150% respectively.

Acceptance criteria: The mean % recovery of the Tranexamic acid and Ethamsylate at each level should be not less than 95.0% and not more than 105.0%.

5. Precision

The precision of the method was demonstrated by intra-day and inter-day precision studies. Intra-day studies were performed by injecting three (3) repeated injections within a day. Peak area and %RSD were calculated and reported. The chromatograms of intra-day precision studies were shown. Inter-day precision studies, was done by injecting three (3) repeated injections for three consecutive days. Peak area and %RSD were calculated and reported.

Intermediate Precision:

Intermediate precision of the analytical method was determined by performing method precision on another day by different analysts under same experimental condition. Assay of all six replicate sample preparations was determined and mean %assay value, standard deviation & %RSD was calculated.

Validation of the method

Linearity: The linearity study was performed for the concentration of 10 ppm to 50 for Tranexamic acid and 60ppm to 300ppm for Ethamsylate and chromatograms are shown below.

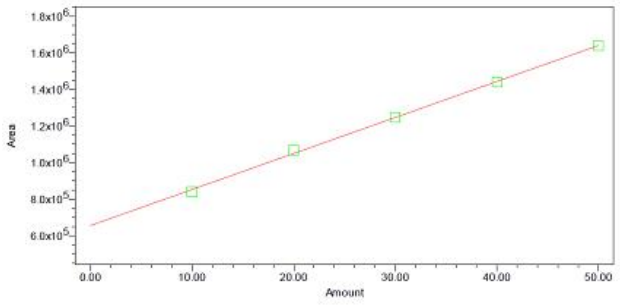

Figure 6: Calibration graph of Tranexamic acid

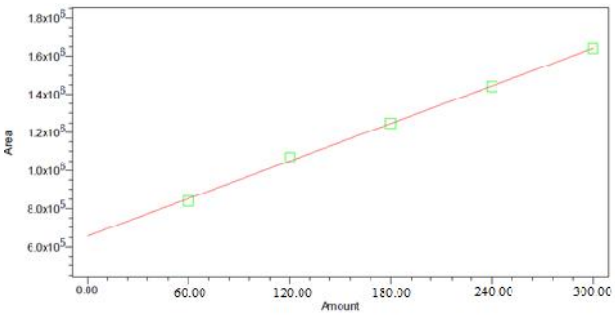

Figure 7: Calibration graph of Ethamsylate

Recovery studies: Sample solutions at different conc. (50%, 100%, and 150%) were prepared and the % recovery was calculated.

Detection limit: The LOD was performed for Tranexamic acid and Ethamsylate was found to be 3.052and 3.402 respectively.

Quantitation Limit

The LOQ was performed for Tranexamic acid and Ethamsylate was found to be 9.85 and 10.12 respectively.

4. Conclusion

A new method was established for simultaneous estimation of Tranaxamic acid and Ethamysylate by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Tranaxamic acid and Ethamsylate by using Thermosil C18 column (4.6×100mm) 5µ, flow rate was 1ml/min, mobile phase ratio was (35:65v/v) Methanol: Phosphate buffer pH 3.0, detection wavelength was 256nm. The instrument used was Waters HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.466 mins and 4.337 mins. The % purity of Tranaxamic acid and Ethamysylate was found to be 99.83% and 98.89% respectively. The system suitability parameters for Tranaxamic acid and Ethamysylate such as theoretical plates and tailing factor were found to be 2095, 1.6 and 2766 and 1.2, the resolution was found to be 5.4. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Tranaxamic acid and Ethamysylate was found in concentration range of 10µg-50µg and 60µg-300µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 98.54% and 100.07%, %RSD for repeatability was 0.36 and 0.46, % RSD for intermediate precision was 0.1 and 0.1 respectively. The precision study was precision, robustness and repeatabilty.LOD value was 3.052 and 3.402 and LOQ value was 9.85 and 10.12 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Tranaxamic acid and Ethamysylate in API and Pharmaceutical dosage form.

Table 1: Calibration data of Tranexamic acid

S.No	Linearity Level	Concentration	Area
1	I	10 ppm	839286
2	II	20 ppm	1067774
3	III	30 ppm	1246474
4	IV	40 ppm	1439994
5	V	50 ppm	1639065
Correlation Coefficient			0.99932

Table 2: Calibration data of Ethamsylate

S.No	Linearity Level	Concentration	Area
1	I	60 ppm	626221
2	II	120 ppm	778750
3	III	180 ppm	931447
4	IV	240 ppm	1070162
5	V	300 ppm	1196060
Correlation Coefficient			0.99916

Table 3: Showing accuracy results for Tranexamic acid

Sample No.	Spike Level	Amount (µg/ml) added	Amount (µg/ml) found	% Recovery	Mean % Recovery
1	50 %	5	4.9	98%	100%
		5	5.1	102%	
		5	5	100%	
2	100 %	10	9.88	98.8%	99.31%
		10	9.91	99.1%	
		10	9.95	99.5%	
3	150 %	15	14.89	99.2%	99.89%
		15	14.86	99.0%	
		15	14.99	99.79%	

Table 3: Showing accuracy results for Ethamsylate

Sample No.	Spike Level	Amount (µg/ml) added	Amount (µg/ml) found	% Recovery	Mean % Recovery
1	50 %	5	4.9	98%	100%
		5	5.1	102%	
		5	5	100%	
2	100 %	10	9.88	98.8%	99.13%
		10	9.91	99.1%	
		10	9.95	99.5%	
3	150 %	15	14.89	99.2%	99.69%
		15	14.86	99.0%	
		15	14.82	99.79%	

Table 4: System Suitability Results for Tranexamic acid

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	3463	1.7
2	*Actual	3452	1.7
3	5 % more	2795	1.6

Table 5: System Suitability Results for Ethamsylate

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	8488	1.3
2	*Actual	4556	1.4
3	5 % more	4931	1.5

Table 6: Precession data of Tranexamic acid and Ethamsylate

Name: Tranexamic acid

	Name	RT	Area	Height (µV)
1	Tranexami	2.453	753403	112688
2	Tranexamil	2.455	748107	113637
3	Tranexamil	2.453	747266	112849
4	Tranexami	2.452	748776	112478
5	Tranexami	2.450	749758	111779
Mean			749462	
Std. Dev.			2384.6	
% RSD			0.32	

Name: Ethamsylate

	Name	RT	Area	Height (µV)
1	Ethamsy	4.289	419183	52411
2	Ethamsy	4.309	416643	52475
3	Ethamsy	4.306	414052	51841
4	Ethamsy	4.300	415235	51804
5	Ethamsy	4.295	416260	51274
Mean			416274	
Std. Dev.			1911.7	
% RSD			0.46	

Table 7: Intermediate precision for Tranexamic acid and Ethamsylate

Name: Tranexamic acid

	Name	RT	Area	Height (µV)
1	Tranexami	2.465	752386	111226
2	Tranexami	2.472	752118	112497
3	Tranexami	2.467	755566	110347
4	Tranexami	2.466	757638	109792
5	Tranexami	2.472	757330	110661
Mean			755008	
Std. Dev.			2638.6	
% RSD			0.35	

Name: Ethamsylate

	Name	RT	Area	Height (µV)
1	Ethamsy	4.323	412252	50991
2	Ethamsy	4.343	408090	50664
3	Ethamsy	4.324	414361	50295
4	Ethamsy	4.323	414692	49813
5	Ethamsy	4.342	411255	49826
Mean			412130	
Std. Dev.			2676.0	
% RSD			0.65	

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