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RESEARCH ARTICLE

Development and Validation of High Performance Thin Layer Chromatographic Method for Simultaneous Estimation of Etamsylate and Tranexamic Acid in Tablet Dosage Form

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ABSTRACT

A simple, novel, rapid, precise, accurate, specific and cost effective High performance thin layer chromatographic method has been developed and validated for simultaneous determination of Etamsylate and Tranexamic Acid in combined tablet dosage form. The stationary phase used was precoated silica gel $60F_{254}$ plate. The mobile phase used was the mixture of water: acetone: methanol (3:4:3v/v/v). The detection of spots was carried out densitometrically using a UV detector at 230nm in absorbance mode. Developed method was validated according to the International Conference on Harmonization (ICH) guidelines. Calibration curve was found to be linear between 2000 to 12000 ng/spot for both drug with correlation coefficient 0.9993 and 0.9988 for Etamsylate and Tranexamic acid respectively. Accuracy in terms of % recovery was found to be 98.95-99.50 and 98.41-99.54 for Etamsylate and Tranexamic acid respectively. Limit of detection for Etamsylate and Tranexamic was found to be 338.60 ng/spot and 440.07ng/spot respectively. Limit of quantitation for Etamsylate and Tranexamic was found to be 1026.07 ng/spot and 1333.55 ng/spot respectively. Thus the proposed method can be successfully applied for simultaneous determination of Tranexamic Acid and Etamsylate in combined tablet dosage form.

KEYWORDS

Etamsylate, Tranexamic acid, High performance thin layer chromatography, Validation

INTRODUCTION

Etamsylate (ETS) is chemically N-ethylamine 2, 5-dihydroxybenzenesulphonate.[1] It is a haemostatic agent.[1] Etamsylate reduce bleeding time and blood loss by increasing platelet aggregation mediated by a thromboxane A2 or prostaglandin F2α dependent mechanism and decreased concentrations of 6-oxoprostaglandin F1α.[2] Etamsylate improves capillary wall stability, but does not stabilize fibrin.[3]

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Etamsylate shown to be effective in reducing blood loss from menorrhagia[4] and after trans-urethral resection of the prostate.[5] Etamsylate is official in British Pharmacopoeia.[6]

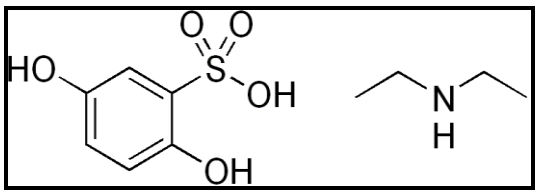

Figure 1: Structure of Etamsylate

Individual determination of Etamsylate is carried out by UV[7], HPLC[8] and Electro

chemiluminescence.[9] A representative structure of Etamsylate was shown in Figure-1.

Tranexamic Acid (TXA) is chemically trans-4-amino methyl-cyclo hexane carboxylic acid.[1] It is an antifibrinolytic agent.[1] Tranexamic Acid reversibly blocking lysine binding sites on plasminogen and thus preventing fibrin degradation.[10] Tranexamic Acid has been used in heavy bleeding associated with uterine fibroids, neoplasms, gastrointestinal bleeding, hematuria, postoperative bleeding.[11] Tranexamic Acid is official in British Pharmacopoeia[6] and Japanese Pharmacopoeia.[12] Individual determination of Tranexamic Acid is carried out by LC[12], UV[13], HPLC[14] and LC-MS/MS.[15] A representative structure of Tranexamic acid was shown in Figure-2.

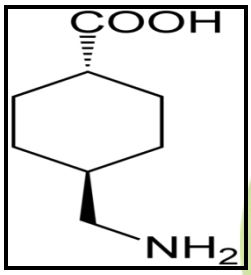

Figure 2: Structure of Tranexamic Acid

ETS and TXA fixed dose combination is used in the management of menorrhagia.[10] The combination therapy is a two pronged approach to control bleeding by the antifibrinolytic action of TXA and achieving hemostasis by improving platelet adhesiveness and restoring capillary resistance by the action of ETS.[10] Literature survey reveals that number of methods such as RP-HPLC[16] and spectrophotometry[17] (simultaneous equation) are reported for simultaneous estimation of ETS and TXA in tablet dosage form. Hence, in the present assay a new simple, accurate, precise and specific HPTLC method is developed and validated for simultaneous estimation of TXA and ETS in tablet formulation.

MATERIAL AND METHODS

Instruments

A Camag HPTLC system (Switzerland) comprising of Camag Linomat V semiautomatic sample applicator, Camag TLC Scanner 3, Camag (Muttenz, Switzerland) flat bottom and twin-trough developing glass chamber (10 × 10 cm), UV cabinet with dual wavelength UV lamp, Camag winCATS 3.2.1 software, Hamilton syringe (100 µl) and weighing balance.

Materials and Reagents

Pure sample of Etamsylate was provided as a gift sample from FDC pharmaceutical limited, Verna industrial estate, Goa, India and Tranexamic Acid was obtained from Jinlan pharma drugs technology co., Limited, Hangzhou, China. A commercial tablet formulation (TRAPIC- E 250mg) was obtained from local market. Silica Gel 60 F254TLC plates were used as stationary phase. All the chemicals used were of analytical grade.

Preparation of Standard and Sample Solution

ETS (50 mg) and TXA (50 mg) were weighed accurately, transferred to 25ml volumetric flask, dissolved and diluted with methanol: water (10:15) to produce 2000µg/ml. Twenty tablets (each containing 250 mg ETS and 250 mg TXA) weighed accurately and ground to fine powder. The powdered equivalent to 50 mg of ETS and 50 mg of TXA was accurately weighed and transferred to a 25ml volumetric flask and made up volume with solvent methanol: water (10:15) to produce 2000µg/ml solution of both drugs.

Chromatographic Conditions

The experiment was performed on silica gel 60F254 aluminum sheets (10 x 10 cm) as stationary phase, using mobile phase comprised of water: acetone: methanol (3:4:3v/v/v). TLC plates were prewashed with methanol and activated in an oven at 50^0 for 5 mins. The solutions were applied on TLC plate in the form of bands of 6 mm width under a stream of nitrogen gas using a Camag Linomat V automatic sample applicator. A constant application rate of 0.1 ml/s was employed and space between two bands was fixed at 5 mm. Ascending development to 80 mm was performed in 10 cm x 10 cm Camag twin trough glass chamber (Muttenz, Switzerland) saturated with the mobile phase for 30 min at room temperature. The

developed TLC plate was air dried and then scanned between 200 to 400 nm using Camag TLC scanner 3 using WinCATS 3.2.1 software. Both components show reasonably good response at 230 nm with scanning speed of 10 mm/s. 3 µl of standard and sample solutions of ETS and TXA were spotted and developed.

Validation of HPTLC Method

The developed method was validated according to the International Conference on Harmonization (ICH) guidelines.[18]

Linearity

Calibration curves were plotted over the concentration range of 2000-12000 ng/spot and 2000- 12000 ng/spot for ETS and TXA, respectively. From the standard solution (2000µg/ml) 1, 2, 3, 4, 5µL volumes were spotted on HPTLC plate. The TLC plate was developed and photometrically analyzed. The calibration curve was prepared by plotting peak area versus concentration (ng/spot) corresponding to each spot. Each reading was an average of 3 determinations.

Method Precision

Repeatability

The precision of the instrument was checked by repeatedly injecting (n = 6) solutions of ETS and TXA without changing the parameters of the proposed method. The results were reported in terms of relative standard deviation (% RSD).

Intermediate Precision

The intraday and interday precisions of the proposed method was determined by estimating the corresponding responses 3 times on the same day and on 3 different days over a period of one week for 3 different concentration of solution of ETS and TXA for the proposed method. The results were reported in terms of relative standard deviation (% RSD).

Accuracy (% Recovery)

The accuracy of the method was determined by calculating recoveries of ETS and TXA by the standard addition method. Known amounts of standard solutions of ETS and TXA was added at 80, 100 and 120 % level to prequantified sample solution of ETS and TXA. The amount of ETS and TXA was estimated by applying obtained values to the respective regression line equations.

Limit of Detection and Limit of Quantitation

The limit of detection (LOD) and limit of quantification (LOQ) were calculated using signal-to-noise (i.e. 3.3 for LOD and 10 for LOQ) ratio using following equations designated:

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where, σ = the standard deviation of the response,

S = slope of the calibration curve.

Robustness

The robustness was studied by evaluating the effect of small but deliberate variations in the chromatographic conditions. The introduction of small changes like change in the mobile phase composition, mobile phase volume and duration of saturation. The effects of these changes on the results were examined.

Specificity

The ability of an analytical method to unequivocally assess the analyte in the presence of other components can be demonstrated by evaluating specificity. The specificity of the HPTLC method was determined by analyzing standard drug and test samples. The spot for ETS and TXA in the samples was confirmed by comparing the R_f and spectrum of the spot to that of a standard. The peak purity of ETS and TXA was determined by comparing the spectrum at three different regions of the spot i.e. peak start (S), peak apex (M) and peak end (E).

Analysis of the Marketed Formulations

Twenty tablets (each containing 250 mg ETS and 250 mg TXA) weighed accurately and ground to fine powder. The powdered equivalent to 50 mg of ETS and 50 mg of TXA was accurately weighed and transferred to a 25ml volumetric flask and made up volume with solvent methanol: water (10:15). 3µl of sample solution

from formulation was applied separately on TLC plate, developed and scanned. The amount of ETS and TXA present in the sample solution was determined by fitting area values of peak corresponding to ETS and TXA into the respective calibration curve.

RESULTS AND DISCUSSION

The TLC procedure was optimized with a view to develop an assay method for the simultaneous estimation of ETS and TXA. The standard solutions of both the drugs were spotted on the TLC plates and run in different solvent systems. The mobile phase consisting of water: acetone: methanol (3:4:3v/v/v) gave sharp and symmetrical peaks with the R_f values of 0.78 and 0.50 for ETS and TXA respectively.

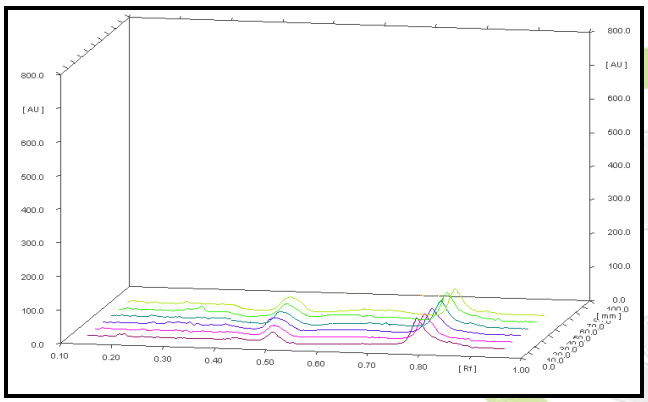

Figure 3: 3-D chromatogram of ETS and TXA in different concentrations at 230 nm

Table 1: Calibration curve data of ETS

Concentration (ng/spot)	Peak area
2000	2464.81
4000	2816.14
6000	3224.76
8000	3637.90
10000	4054.27
12000	4467.22

Well defined spots were obtained when the chamber was saturated with mobile phase for 30 min at room temperature. A 3-D chromatogram showing peaks of ETS and TXA in different concentrations at 230 nm are depicted in Figure 3.

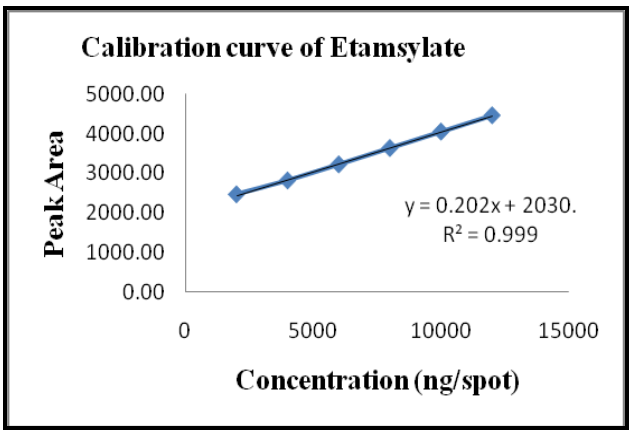

Figure 4: Calibration Curve for Etamsylate (2000-12000 ng/spot)

Table 2: Calibration curve data of Tranexamic acid

Concentration (ng/spot)	Peak area
2000	1049.91
4000	1273.83
6000	1519.13
8000	1792.92
10000	2039.01
12000	2250.09

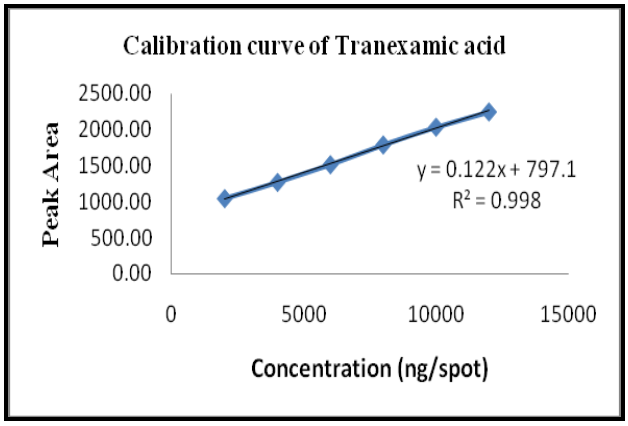

Figure 5: Calibration Curve for Tranexamic acid (2000-12000 ng/spot)

Table 3: Repeatability data for ETS and TXA

Concentration ng/spot		Peak Area		Mean ± S.D n= 6		%R.S.D	
ETS	**TXA**	**ETS**	**TXA**	**ETS**	**TXA**	**ETS**	**TXA**
2000	2000	2464.83	1050.93	2463.81± 3.91	1050.89 ± 3.59	0.16	0.34
2000	2000	2462.15	1048.88				
2000	2000	2467.02	1056.42				
2000	2000	2460.96	1046.13				
2000	2000	2469.16	1053.27				
2000	2000	2458.76	1049.68				

Table 4: Intraday precision data for ETS and TXA

Concentration taken (ng/spot)		% Estimated		Mean ± S.D (n=3)		%RSD	
ETS	**TXA**	**ETS**	**TXA**	**ETS**	**TXA**	**ETS**	**TXA**
4000	4000	99.74	98.20	99.26 ±0.66	98.29 ±0.14	0.67	0.15
4000	4000	98.51	98.22				
4000	4000	99.54	98.46				
6000	6000	99.13	97.96	98.78 ±0.43	98.33 ±0.36	0.44	0.37
6000	6000	98.30	98.33				
6000	6000	98.91	98.69				
8000	8000	99.49	99.88	99.23 ±0.27	99.21 ±0.65	0.28	0.66
8000	8000	99.26	99.19				
8000	8000	98.94	98.57				

Table 5: Interday precision data for ETS and TXA

Concentration taken (ng/spot)		% Estimated		Mean ± S.D (n=3)		%RSD	
ETS	**TXA**	**ETS**	**TXA**	**ETS**	**TXA**	**ETS**	**TXA**
4000	4000	99.98	98.58	99.23 ±0.71	99.05 ±0.73	0.72	0.74
4000	4000	98.55	99.90				
4000	4000	99.16	98.67				
6000	6000	99.00	98.73	98.98 ±0.59	98.88 ±0.93	0.60	0.94
6000	6000	98.37	98.04				
6000	6000	99.56	99.88				
8000	8000	99.66	99.59	99.97 ±1.01	98.78 ±0.73	1.02	0.74
8000	8000	101.11	98.58				
8000	8000	99.15	98.17				

Table 6: Accuracy data for ETS and TXA

Assay level (%)	**Tablet powder taken eq to (mg)**		**Standard added (mg)**		**Total drug recoverd (mg)**		**%Recovery of standard added**		**Mean ± S.D (n=3)**	
	ETS	**TXA**	**ETS**	**TXA**	**ETS**	**TXA**	**ETS**	**TXA**	**ETS**	**TXA**
Blank	50	50	0	0	48.73	49.94	-	-	-	-
	50	50	0	0	49.09	48.64	-	-	-	-
	50	50	0	0	49.32	48.28	-	-	-	-
					49.05	**48.95**	-	-	-	-
80	50	50	40	40	88.85	88.11	99.52	97.89	99.01 ± 0.67	98.94 ± 1.02
	50	50	40	40	88.35	88.56	98.25	99.02		
	50	50	40	40	88.75	88.92	99.27	99.92		
100	50	50	50	50	98.96	98.29	99.82	98.68	98.95 ± 0.80	99.54 ± 1.00
	50	50	50	50	98.17	99.27	98.25	100.64		
	50	50	50	50	98.42	98.61	98.76	99.31		
120	50	50	60	60	108.87	108.74	99.70	99.64	99.50 ± 0.89	98.41 ± 0.59
	50	50	60	60	109.20	108.20	100.26	98.74		
	50	50	60	60	108.16	108.87	98.53	99.85		

Table 7: Robustness of ETS and TXA

Change Parameters		**Peak Area**		**Mean ± S.D n= 3**		**%R.S.D**	
		ETS	**TXA**	**ETS**	**TXA**	**ETS**	**TXA**
Mobile phase composition (Water : Acetone : Methanol)							
1	3:4:3 v/v/v	3225.63	1511.87	3215.66 ± 9.43	1503.36 ± 8.14	0.29	0.54
2	4:3:3 v/v/v	3206.88	1495.64				
3	3:3:4 v/v/v	3214.48	1502.59				
Mobile phase volume							
1	10 ml	3229.43	1516.75	3219.00 ± 9.53	1510.67 ± 6.14	0.30	0.41
2	12 ml	3210.73	1504.47				
3	15 ml	3216.86	1510.79				
Saturation time							
1	15 min	3227.36	1513.28	3218.21 ± 8.83	1505.61 ± 7.24	0.27	0.48
2	20 min	3209.74	1498.89				
3	30 min	3217.53	1504.67				

Table 8: Analysis of marketed formulation of ETS and TXA

Tablet powder taken eq to (mg)		**Amount found (mg/tablet)**		**% Estimated**		**Mean (%Estimated) ± S.D (n=3)**	
ETS	TXA	ETS	TXA	ETS	TXA	ETS	TXA
50	50	49.30	49.72	98.61	99.44	99.05 ±0.43	98.72 ±0.63
50	50	49.53	49.16	99.07	98.32		
50	50	49.74	49.20	99.48	98.40		

Table 9: Summary of validation parameter

Parameter	**HPTLC Method**	
	ETS	**TXA**
Linearity range (ng/spot)	2000-12000	2000-12000
Corelation Coefficeint	0.9993	0.9988
Repeatability (%RSD) (n=6)	0.16	0.34
Intraday Precision (%RSD) (n=3)	0.28-0.67	0.15-0.66
Interday Precision (%RSD) (n=3)	0.60-1.02	0.74-0.94
% Assay	99.05	98.72
Accuracy (%Recovery)	98.95-99.50	98.41-99.54
LOD (ng/spot)	338.60	440.07
LOD (ng/spot)	1026.07	1333.55

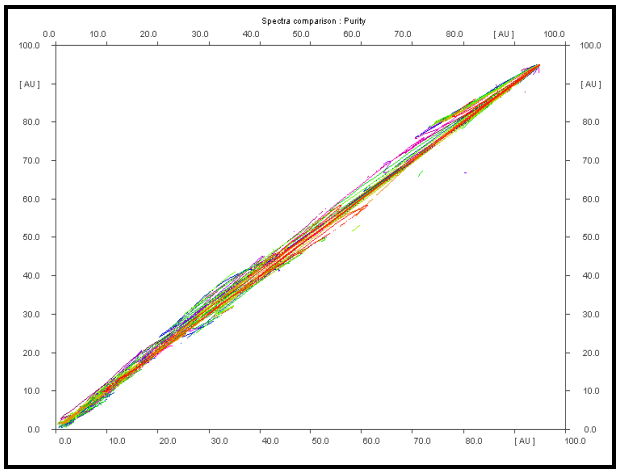

Figure 6: Specificity of ETS and TXA

The proposed HPTLC method was validated in terms of linearity, precision, accuracy, LOD, LOQ, robustness and specificity. The calibration plot was found to be linear over the concentration range 2000-12000 and 2000-12000 ng/spot for ETS and TXA, respectively with a correlation coefficient of 0.9993 and 0.9988 for ETS and TXA, respectively. Relative standard deviation for repeatability of measurements is less than 2% (0.16 for ETS and 0.34 for TXA), which indicates that the proposed method is repeatable (Table 3). The low % RSD values (less than 2) of intraday (0.28-0.67for ETS and 0.15-0.66 for TXA) (Table 4) and interday (0.60-1.02 for ETS

and 0.74-0.94 for TXA) (Table 5) precision reveals that the proposed method is precise. To study the accuracy of the method, recovery studies were performed. The percent average recoveries obtained were 98.95-99.50 and 98.41-99.54 for ETS and TXA, respectively indicating that the proposed HPTLC method is highly accurate (Table 6). The % RSD for all the parameters in the robustness study are found to be less than 2% indicates robustness of the method (Table 7). The proposed validated method was successfully applied to determine ETS and TXA in tablet dosage forms. The percent average assay was found to be 99.05 and be 98.72 for ETS and TXA, respectively (Table 8). LOD for ETS and TXA were found to be 338.60 ng/spot and 440.07 ng/spot, respectively (Table 9). LOQ for ETS and TXA were found to be 1026.07 ng/spot and 1333.55 ng/spot, respectively (Table 9). To confirm the specificity of the proposed method, the solution of formulation was spotted on TLC plate, developed and scanned. It was observed that the excipients present in the formulation did not interfere with the sample peak (Figure 6).

CONCLUSION

It could be concluded from the results obtained in the present investigation that the above developed and validated method for the simultaneous estimation of TXA and ETS in tablet dosage form was novel, simple, accurate, and precise and can be used, successfully in other routine laboratory analysis.

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