International Journal of ChemTech Research
CODEN( USA): IJCRGG ISSN : 0974-4290
Vol.2, No.1, pp 74-78, Jan-Mar 2010

# Spectrophotometric Methods for Simultaneous Estimation of Ethamsylate and Tranexamic Acid from Combined Tablet Dosage Form

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**ABSTRACT:** A simple, rapid, accurate, precise, specific and economical spectrophotometric method for simultaneous estimation of Ethamsylste and Tranexamic acid in combined tablet dosage form has been developed. The developed method employs derivatization procedure for making tranexamic acid UV detectable. It employs formation and solving of simultaneous equation using two wavelengths 299.0 nm and 286.2 nm. This method obeys Beer's law in the employed concentration ranges of 4-15 µg $mL^{-1}$ and 2-12 µg $mL^{-1}$ for Ethamsylate and Tranexamic acid respectively. Results of analysis were validated statistically and by recovery studies.
**KEY WORDS:** Ethamsylste, Tranexamic acid, Simultaneous Equation, Derivatization

## INTRODUCTION

Ethamsylste (ESL) is chemically, 2, 5 – dihydroxy benzenesulfonic acid with diethylamine[1], belongs to the class of haemostatic compound that inhibits prostacycline synthetase, an enzyme which converts arachidonic acid to Prostacycline and thereby enhances platelet aggregation and platelet adhesiveness. It is used for the prevention and treatment of capillary hemorrhage, hematemesis, hemopthsis, malena, hematuria, epistaxis, menorrhagia and postpartum hemorrhage[2]. Ethamsylste is official in british pharmacopoeia[3].

Tranexamic acid (TA) is chemically trans-4-aminomethyl-cyclohexaecarboxylic acid[1]. It competitively inhibits activation of plasminogen, 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. It is used for controlling abnormal bleeding in a number of diseases[2]. Tranexamic acid is official in British Pharmacopeia[3]. Tranexamic acid combination with Ethamsylste has been used for prevention of bleeding after surgery or trauma, bleeding of subarachnoid hemorrhage, primary or intrauterine contraceptive device (IUCD) induced menorrhagia. Spectrophotometric methods are reported, the individual and in combination for estimation of Tranexamic acid and Ethamsylste in the tablet dosage form[4-11]. HPLC methods are reported, the individual and in combination for estimation of Tranexamic acid and Ethamsylste in the tablet dosage form [12-15]. Tranexamic acid is not having any conjugation in its structure i.e., lack of chromophore. Hence it is essential to make derivatives of tranexamic acid to make it UV detectable.

## MATERIALS AND METHODS

### Apparatus:

Shimadzu double beam UV–visible spectrophotometer with 10 mm matched quartz cell model UV 1800 (Japan) was used for the development of proposed method.

**Reagents and Solutions:**
Ethamsylste and Tranexamic acid were kindly gifted from Mercury Laboratories Ltd., (Baroda). ETOSYS (Systopic Laboratories) having content Ethamsylste-250 mg and Tranexamic acid-250 mg was purchased from local market. All the chemicals and reagents were of A.R grade and purchased from Merck ltd, Mumbai.

**Solvent System:**
11.0 g of sodium dihydrogen phosphate was dissolved in 600 ml of distilled water and 400 ml of methanol was added to it.

**Derivatization Procedure:**
The standard stock solution of ESL and TA were prepared by dissolving 10 mg each drug in solvent system in 100 ml volumetric flask to give stock solution having concentration of 100 µg /ml. Then several working standard solutions were prepared by transferring required aliquots of standard drug solutions in 10 ml volumetric flask with addition of 4 ml of glacial acetic acid to each and volume is made up to 10 ml with solvent system. All dilutions were scanned in wavelength range of 400 nm to 200 nm. The λ-max of Ethamsylate and Tranexamic acid were found to be 299.0 nm and 286.2 nm respectively.

**Procedure for Calibration Curve:**
Standard solutions of Ethamsylate in the concentration range of 4 µg/ml to 15µg/ml obtained by transferring (0.4, 0.6, 0.8, 1.0, 1.2, 1.5 ml) of Ethamsylate stock solution (100 µg/ml) to the series of 10 ml volumetric flasks and standard solutions of tranexamic acid in the concentration range of 2 µg/ml to 12 µg/ml were obtained by transferring (0.2, 0.4, 0.6, 0.8, 1.0, 1.2 ml) of Tranexamic acid stock solution (100 ppm) to the series of 10 ml volumetric flasks. Then 4 ml glacial acetic acid was added to each volumetric flask and volume was made up to 10 ml with solvent system. All dilutions were scanned in wavelength range of 400 nm to 200 nm. The absorbances were plotted against the respective concentrations to obtain the calibration curves. A representative overlain spectrum of Ethamsylste and Tranexamic acid in solvent system is shown in Fig 1.

**Formation of Simultaneous Equation:**
Set of two simultaneous equations were:
$Cx = (A_2\, ay_1 - A_1\, ay_2)/ (ax_2\, ay_1 - ax_1\, ay_2)$ and
$Cy = (A_1\, ax_2 - A_2 ax_1)/ (ax_2\, ay_1 - ax_1\, ay_2)$
Where $A_1$ and $A_2$ are the absorbance of sample solutions at 299.0 nm and 286.2 nm respectively. Cx and Cy are concentration of Ethamsylste and Tranexamic acid in mg/mL in sample solution. By substituting the values of $A_1$ and $A_2$ the values of Cx and Cy can be calculated by solving the two equations simultaneously. Here, $a_{x1}$ and $a_{x2}$ are the absorptivity coefficient of Ethamsylste at 299.0 nm and 286.2 nm respectively, $a_{y1}$ and $a_{y2}$ are the absorptivity coefficient of Tranexamic acid at 299.0 nm and 286.2 nm respectively.
The equations were formed as follows:
$Cx = ( A_2\, 49.96 – A_1\, 60.36)/ -1690.95$ and
$Cy = (A_1\, 12.62 – A_2\, 38.46)/ -1690.95$
The optical parameters & regression characteristic for Ethamsylste and Tranexamic acid are shown in Table 1.

**Method Validation**
From validation studies it was found that the developed method is specific as percentage interference was found to be -0.426 and -0.275 for Ethamsylste and Tranexamic acid respectively. The linearity range for Ethamsylste and Tranexamic acid were 4-15 µg $mL^{-1}$ and 2-12 µg $mL^{-1}$ respectively.
Recovery studies was carried out by addition of standard drug solution to pre-analysed tablet sample solution at three different concentration levels taking into consideration percentage purity of added bulk drug sample. The results of the recovery studies are found to be satisfactory and shown in Table 2. The results obtained from recovery study (accuracy study) indicated that mean of percentage recovery were 100.411 ± 1.616 and 99.946 ± 0.670 for Ethamsylste and Tranexamic acid respectively.
Repeatability studies were found to be satisfactory with % RSD 1.445 and 0.914 for Ethamsylste and Tranexamic acid respectively. Intraday studies showed % RSD 1.239 and 0.763 for Ethamsylste and Tranexamic acid respectively. Interday studies showed % RSD 1.742 and 0.784 for Ethamsylste and Tranexamic acid respectively. The results of Intra and Inter day studies are shown in Table 3.
The limit of detection (LOD) was calculated to be 0.3202 µg $mL^{-1}$ and 0.1746 µg $mL^{-1}$ for Ethamsylste and Tranexamic acid respectively. The limit of quantification (LOQ) was calculated to be 3.785 µg $mL^{-1}$ and 1.231 µg $mL^{-1}$ for Ethamsylste and Tranexamic acid respectively.

**Estimation of Ethamsyalte and Tranexamic acid in Pharmaceutical Tablets:**
Twenty tablets were accurately weighed and average weight of content per tablet was calculated. The contents of tablet were reduced to fine powder and mixed thoroughly. A quantity of tablet powder equivalent to 20 mg was transferred to 100 ml volumetric flask and mixed with 70 ml of solvent system. The solution was sonicated for 10 minutes, there after volume was made up to 100 ml with same solvent system. The solution was filtered through Whatman filter paper no. 41. Then 0.8 ml from above stock solution was transferred to 10 ml volumetric flask, 4 ml glacial acetic acid was added and volume

was made up to 10 ml with solvent system. The absorbance of sample solution was measured at 299.0 nm and 286.2 nm against blank. The content of ESL and TA in tablet was calculated using two framed simultaneous equations and results of analysis are shown in Table 4.

## RESULTS AND DISCUSSION

The proposed method for simultaneous estimation of Ethamsylste and Tranexamic acid in combined dosage form were found to be simple, rapid, accurate, precise, specific and economical. Since none of the method is reported for simultaneous analysis of the two drugs earlier, the developed method can be used for routine analysis of two drugs in combined dosage forms.

The method involving formation and solving of simultaneous equation is very simple for routine analysis of two drugs in combined dosage forms. Once the equations are formed, then only measurement of the absorbance of sample solution at two wavelengths and simple calculations are required.

**Table 1: Optical parameters & regression characteristic for Ethamsylste and Tranexamic acid**

| Parameters | Ethamsylate | | Tranexamic acid | |
|---|---|---|---|---|
| | 286.2 nm | 299 nm | 286.2 nm | 299 nm |
| Beers's law limit (µg/ml) | 4-15 | 4-15 | 2-12 | 2-12 |
| Molar absorptivity ($l\ mole^{-1}cm^{-1}$) | $3.323 \times 10^3$ | $1.012 \times 10^3$ | $9.489 \times 10^3$ | $7.854 \times 10^3$ |
| Sandell's sensitivity ($mg/cm^2$/.001absorbance unit) | 0.07923 | 0.026001 | 0.016567 | 0.020016 |
| Regression equation (y= a + bc)<br>slope (b)<br>intercept (a) | <br>0.0201<br>-0.0570 | <br>0.0334<br>0.0378 | <br>0.0648<br>-0.0214 | <br>0.0633<br>-0.0669 |
| Correlation coefficient ($r^{2)}$ | 0.9986 | 0.9989 | 0.9992 | 0.9988 |

**Table 2: Results of recovery studies**

| Conc. Added (mcg) | | % Conc. recovered | | Mean Recovery ±S.D* | |
|---|---|---|---|---|---|
| ESL | TA | ESL | TA | ESL | TA |
| 2 | 2 | 102.28 | 100.58 | 100.52 ± 1.5950 | 99.94 ± 0.5565 |
| 4 | 4 | 99.17 | 99.67 | | |
| 6 | 6 | 100.11 | 99.57 | | |

*Average of three determinations

**Table 3: Inter-day and Intra-day precision**

| Inter-day | | | | | | Intra-day | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Amount taken* | | Amount found ±S.D** | | %RSD | | Amount taken* | | Amount found ±S.D** | | %RSD | |
| ESL | TA | ESL | TA | ESL | TA | ESL | TA | ESL | TA | ESL | TA |
| 6.4 | 6.4 | 6.276± 0.114 | 6.186± 0.063 | 1.812 | 1.020 | 6.4 | 6.4 | 6.307± 0.098 | 6.264± 0.054 | 1.553 | 0.865 |
| 8 | 8 | 8.014± 0.133 | 7.900± 0.050 | 1.666 | 0.634 | 8 | 8 | 8.070± 0.103 | 7.882± 0.066 | 1.286 | 0.837 |
| 9.6 | 9.6 | 9.302± 0.163 | 9.588± 0.067 | 1.747 | 0.699 | 9.6 | 9.6 | 9.262± 0.081 | 9.593± 0.056 | 0.870 | 0.588 |
| **Average %RSD** | | | | **1.742** | **0.784** | **Average %RSD** | | | | **1.239** | **0.763** |

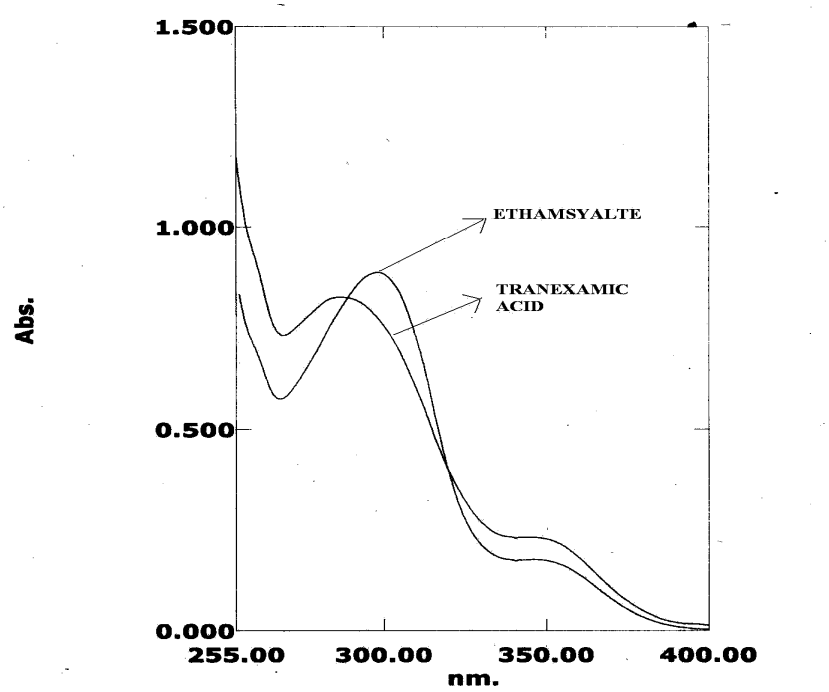
*Concentration in µg
** Average of three determinations

**Table 4: Results of analysis of tablet**

| Formulation | Label claim[a] | | Amount Found (mg) | | %Recovery ± SD[b] | | %RSD | |
|---|---|---|---|---|---|---|---|---|
| | ESL | TA | ESL | TA | ESL | TA | ESL | TA |
| ETOSYS TAB | 250 | 250 | 247.844 | 247.242 | 99.14± 0.471 | 98.90± 0.139 | 0.475 | 0.141 |

[a]Amount in mg
[b]Mean ± Standard Deviation for three determinations

**Fig.1: Overlain spectra of Ethamsylste and Tranexamic acid**



**ACKNOWLEDGEMENTS**

Authors are greatly thankful to Lachoo Memorial College of Science and Technology, Pharmacy Wing, Jodhpur, Rajasthan for providing free access to their facilities to carry out research work. We are also thankful to Mercury Laboratories Ltd., (Baroda) for providing us the free gift samples of Ethamsylste and Tranexamic acid respectively which were required for our research work.

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