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# Simultaneous Estimation of Mefenamic Acid, Ethamsylate and Tranexamic Acid in Bulk and Pharmaceutical Formulations by RP-HPLC Method

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## ABSTRACT

A simple, new, precise, accurate and reproducible RP-HPLC method for simultaneous estimation of mefenamic acid, ethamsylate and tranexamic acid in bulk and pharmaceutical formulations. Separation of mefenamic acid, ethamsylate and tranexamic acid was successfully achieved on a Kromasil C8 (250 mm x 4.6mm x 5µ ) in an isocratic mode utilizing Ammonium acetate buffer and methanol (60:40 *v/v*) at a flow rate of 1.0 mL/min. The method was validated according to ICH guidelines for linearity, sensitivity, accuracy, precision, specificity and robustness. The response was found to be linear in the drug concentration range of 25-75 mg/mL for mefenamic acid ,25-75 mg/mL for ethamsylate 50-150 mg/mL for tranexamic acid. The correlation coefficient was found to be 0.9997 for both the drugs. The limit of detection (LOD) was 0.158,0.2183 and 0.321 for mefenamic acid, ethamsylate and tranexamic acid respectively. The limit of quantification (LOQ) was 0.527,0.7278 and 1.071 for mefenamic acid, ethamsylate and tranexamic acid respectively. The relative standard deviation (RSD) of six replicates is less than 2%. This HPLC method is applied successfully to the simultaneous quantitative analysis of mefenamic acid, ethamsylate and tranexamic acid in commercial tablets.

**Keywords:** RP-HPLC, mefenamic acid, ethamsylate and tranexamic acid, pharmaceutical formulation, analysis

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## INTRODUCTION

Mefenamic acid[1,2] is a member of the anthranilic acid derivatives (or fenamate) class of NSAID drugs and is used to treat mild to moderate pain, including menstrual pain, and is sometimes used to prevent migraines associated with menstruation Chemically, mefenamic acid is described as 2-[(2,3-dimethylphenyl)amino]benzoic acid. Mefenamic acid binds the prostaglandin synthetase receptors COX-1 and COX-2, inhibiting the action of prostaglandin synthetase. As these receptors have a role as a major mediator of inflammation and/or a role for prostanoid signaling in activity-dependent plasticity, the symptoms of pain are temporarily reduced.. The chemical structure of the mefenamic acid is shown in Figure 1.

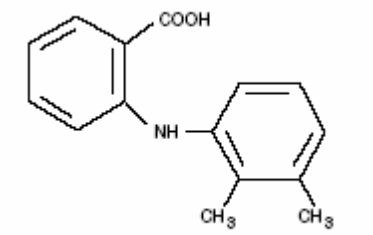


**Figure 1: Chemical structure of mefenamic acid**

Ethamsylate[3,4] is a haemostatic drug. Chemically, ethamsylate is described as 2,5-dihydroxybenzenesulfonic acid; N-ethylethanamine. It stimulates thrombopoiesis and their release from bone marrow. Haemostatic action is due to activation of thromboplastin formation on damaged sites of small blood vessels and decrease of PgI2 (Prostacyclin I2) synthesis; it also facilitates platelet aggregation and adhesion, that at last induce decrease and stop of hemorrhage. The chemical structure of the ethamsylate is shown in Figure 2.

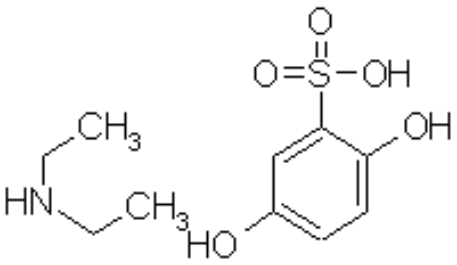


**Figure 2: Chemical structure of ethamsylate**

Tranexamic acid[5,6] is a synthetic analog of the amino acid lysine. Chemically, tranexamic acid is described as (1r,4r)-4-(aminomethyl)cyclohexane-1-carboxylic acid. 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. The chemical structure of the tranexamic acid is shown in Figure 3.

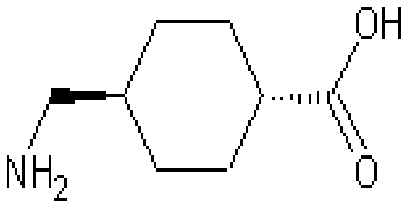


**Figure 3: Chemical structure of tranexamic acid**

The literature reports, many methods for simultaneous quantitative determination of Mefenamic acid, ethamsylate and tranexamic acid in bulk, tablet dosage form, capsule dosage form and human plasma. These methods include simultaneous estimation of Mefenamic acid, ethamsylate and tranexamic acid by UV spectrophotometry[7-11] and HPLC[12,13,14]. The aim of the present investigation is to develop and validate a sensitive, precise and accurate RP-HPLC method for the simultaneous quantification of mefenamic acid, ethamsylate and tranexamic acid in bulk and in its combined pharmaceutical formulation. The proposed method is validated as per ICH guidelines[15].

## MATERIALS AND METHOD

### Chemicals and Reagents

Mefenamic acid, ethamsylate and tranexamic acid were obtained as a gift sample from Lara drugs pvt Ltd., Hyderabad. Ammonium acetate and methanol of HPLC grade was purchased from Merck (India) Ltd., Mumbai. Ortho phosphoric acid of analytical reagent grade was obtained from Sd Fine Chemicals Ltd., Mumbai. Mille Q water was used through out the process.

### Chromatographic apparatus and conditions

The development and validation of the assay was performed on HPLC system with Waters 2695 alliance with binary HPLC pump, Waters 2998 PDA detector, Waters Empower2 software. The analytical column used to achieve chromatographic separation was Kromasil C8, (250 mm × 4.6 ; 5µm) column. The mobile phase consisting of ammonium acetate buffer (pH 4.8) and methanol was degassed and pumped from the solvent reservoir in the ratio of 60:40 *v/v*. The flow rate was 1.0 mL/min. The column temperature was maintained at 30°C. The detection was performed at 240 nm and the run time was 7 min. Injection was carried out using a 10 µL loop. Prior to injection of the drug solution the column was equilibrated for at least 10 minutes with the mobile phase.

**Standard Solution**

250mg mefenamic acid,250mg ethamsylate and 500mg tranexamic acid was accurately weighed, dissolved in mobile phase and diluted to volume in a 100 mL volumetric flask. Pipette out 2.0 mL of the above standard stock into 100 mL volumetric flask and dilute to volume with mobile phase.

**Sample Solution**

Accurately weigh 1322mg of sample. Transfer the sample powder into 100 mL volumetric flask. Add 10 mL mobile phase and sonicate for 20 minutes. The resulting solution was made up to the volume with mobile phase. Filter through the 0.45 µm filter paper. Transfer 2 mL of the above solution into a 100 mL volumetric flask and made up to the volume with mobile phase.

**Method Validation**

**System Suitability**

System suitability tests are an integral part of liquid chromatographic method. System suitability was checked on each day of validation to evaluate the analytical system in order to show that the performance of the system meet the standards required by the method. System suitability parameters established are number of theoretical plates, resolution and tailing factor.

**Linearity and Range**

Linearity was evaluated by analyzing five concentrations of mefenamic acid, ethamsylate and tranexamic acid by the developed method. For linearity and range testing, stock solutions of Mefenamic acid, ethamsylate and tranexamic acid were prepared. Appropriate quantities of these stock solutions were mixed and diluted in a series of volumetric flasks to contain both the drugs in the concentration range of 25-75 mg/mL for mefenamic acid, 25-75 mg/mL for ethamsylate 50-150 mg/mL for tranexamic acid.

**Precision**

The precision of the proposed method was performed by analyzing six sample solutions. The response factor of drug peaks and percentage RSD were calculated.

**Accuracy**

The accuracy of the method was determined through recovery experiments. The accuracy of the proposed method was demonstrated by preparing samples spiked with 50%, 100%, and 150% of the test concentration of Mefenamic acid, ethamsylate and tranexamic acid. Each concentration level was analyzed. Mean percent recovery and percent RSD were calculated for each concentration.

**Robustness**

The robustness test was performed by deliberately making the changes in chromatographic

conditions. Retention time, tailing factor, resolution, and theoretical plates were measured to demonstrate the robustness of the method.

**Limit of detection (LOD), limit of quantification (LOQ):**

The Limit of quantification and detection determines the sensitivity of the method. The LOD and LOQ were calculated using the following formulas (a) and (b).

(a) LOQ = 10 σ / S

(b) LOD = 3.3 σ / S

Where σ = residual standard deviation of response; S = slope of the calibration curve.

## RESULTS AND DISCUSSION

**System Suitability Studies**

The column efficiency, resolution and tailing factor were calculated for the standard solutions (Table 1). The values obtained demonstrated the suitability of the system for the analysis of the selected drug combinations. System suitability parameters may fall within ±2 % Relative standard deviation range during routine performance of the method.

**Table 1: System Suitability**

| Parameter | Mefenamic acid | Ethamsylate | Tranexamic acid |
|---|---|---|---|
| Retention time | 1.854 | 3.148 | 4.416 |
| Theoretical plates | 3137 | 3853 | 3907 |
| Tailing factor | 1.48 | 1.17 | 1.13 |
| % RSD | 0.7 | 0.6 | 0.7 |

**Linearity and range**

The linearity of the method was determined at five concentration levels. The calibration curve was constructed by plotting response factor against concentration of drugs.

Mefenamic acid, ethamsylate and tranexamic acid exhibited linearity of the concentration range of 25-75 µg/mL, 25-75 µg/mL and 50-150 µg/mL (Figures 4, 5 and 6).

The regression equations for the selected drugs are:

Mefenamic acid: y = 38855x - 16146 (R2 = 0.9999)

Ethamsylate : y = 34764x -20725 (R2 = 0.9999)

Tranexamic acid : y = 78575x - 23170 (R2 = 0.9999)

Where y = peak area and x = concentration of the drug in µg/mL

The results show an excellent correlation exists between areas and concentration of drugs. The results for calibration data are shown in Table 2 and calibration curves are given in Figure 4, 5 and 6.

**Table 2: Linearity data of mefenamic acid, ethamsylate and tranexamic acid**

| Mefenamic acid | | Ethamsylate | | Tranexamic acid | |
|---|---|---|---|---|---|
| **Area** | **Amount of drug (µg/mL)** | **Area** | **Amount of drug (µg/mL)** | **Area** | **Amount of drug (µg/mL)** |
| 1935462 | 25 | 1733306 | 25 | 3913385 | 50 |
| 2900284 | 37.50 | 2560915 | 37.5 | 5871029 | 75.00 |
| 3875737 | 50.00 | 3463292 | 50 | 7836025 | 100.00 |
| 4815864 | 62.5 | 4323171 | 62.5 | 9791158 | 125 |
| 5819589 | 75 | 5197673 | 75.00 | 11790243 | 150 |

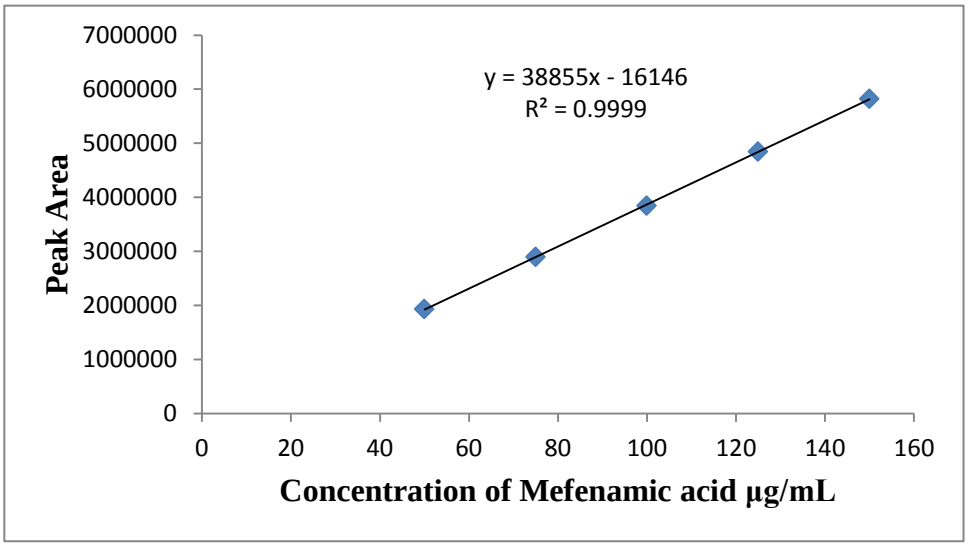


**Figure 4: Linearity curve for mefenamic acid**

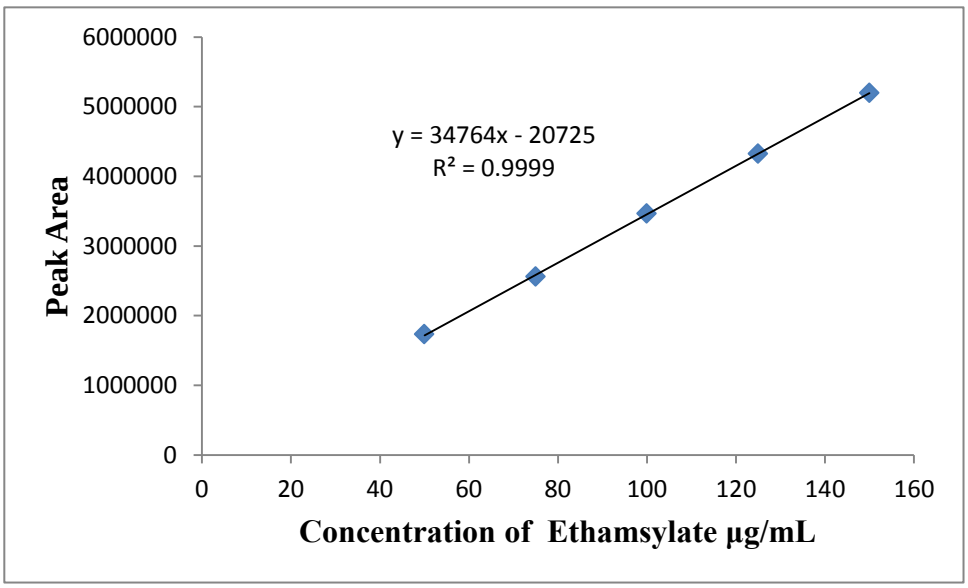


**Figure 5: Linearity curve for ethamsylate**

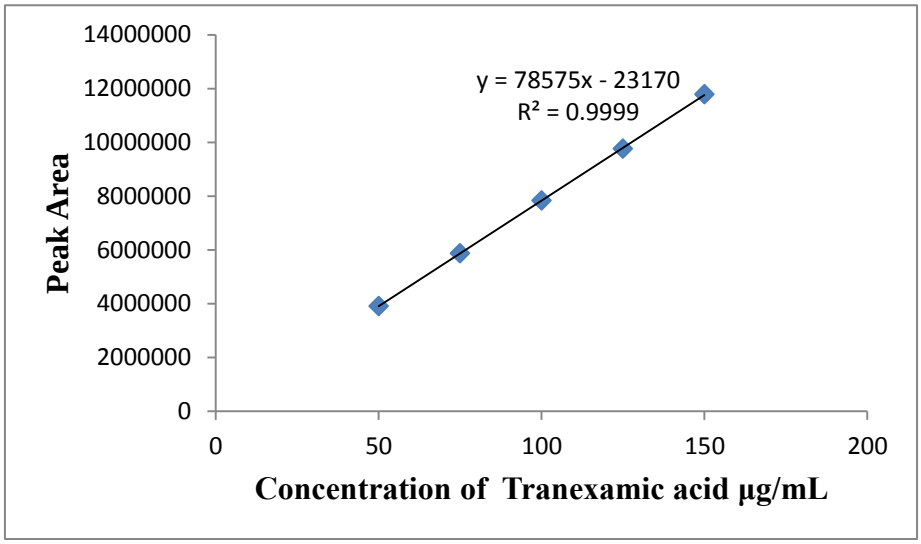


**Figure 6: Linearity curve for tranexamic acid**

**Accuracy and Precision**

The results of accuracy of proposed methods at three different concentration levels are summarized in Tables 3, 4 and 5. The chromatograms at three different levels are shown in Figures 7, 8 and 9. From the results obtained, added recoveries of standard drugs were found to be accurate.

**Table 3: Accuracy for mefenamic acid**

| Accuracy level | Sample weight | µg/mL added | µg/mL found | % Recovery | % Mean |
|---|---|---|---|---|---|
| 50% | 661.00 | 24.750 | 24.82 | 100 | 100 |
| | 661.00 | 24.750 | 24.89 | 101 | |
| | 661.00 | 24.750 | 24.78 | 100 | |
| 100% | 1322.00 | 49.500 | 49.71 | 100 | 100 |
| | 1322.00 | 49.500 | 49.75 | 101 | |
| | 1322.00 | 49.500 | 49.69 | 100 | |
| 150% | 1983.00 | 74.250 | 74.56 | 100 | 100 |
| | 1983.00 | 74.250 | 74.59 | 100 | |
| | 1983.00 | 74.250 | 74.66 | 101 | |

**Table 4: Accuracy for ethamsylate**

| Accuracy level | Sample weight | µg/mL added | µg/mL found | % Recovery | % Mean |
|---|---|---|---|---|---|
| 50% | 661.00 | 25.000 | 24.91 | 100 | 100 |
| | 661.00 | 25.000 | 24.90 | 100 | |
| | 661.00 | 25.000 | 24.96 | 100 | |
| 100% | 1322.00 | 50.000 | 49.89 | 100 | 100 |
| | 1322.00 | 50.000 | 49.92 | 99 | |
| | 1322.00 | 50.000 | 49.81 | 100 | |
| 150% | 1983.00 | 75.000 | 74.81 | 100 | 100 |
| | 1983.00 | 75.000 | 74.80 | 100 | |
| | 1983.00 | 75.000 | 74.78 | 100 | |

**Table 5: Accuracy for tranexamic acid**

| Accuracy level | Sample weight | µg/mL added | µg/mL found | % Recovery | % Mean |
|---|---|---|---|---|---|
| 50% | 661.00 | 49.500 | 49.49 | 100 | 100 |
| | 661.00 | 49.500 | 49.48 | 100 | |
| | 661.00 | 49.500 | 49.52 | 100 | |
| 100% | 1322.00 | 99.000 | 99.02 | 100 | 100 |
| | 1322.00 | 99.000 | 99.08 | 100 | |
| | 1322.00 | 99.000 | 99.02 | 100 | |
| 150% | 1983.00 | 148.500 | 148.86 | 100 | 100 |
| | 1983.00 | 148.500 | 148.81 | 100 | |
| | 1983.00 | 148.500 | 148.32 | 100 | |

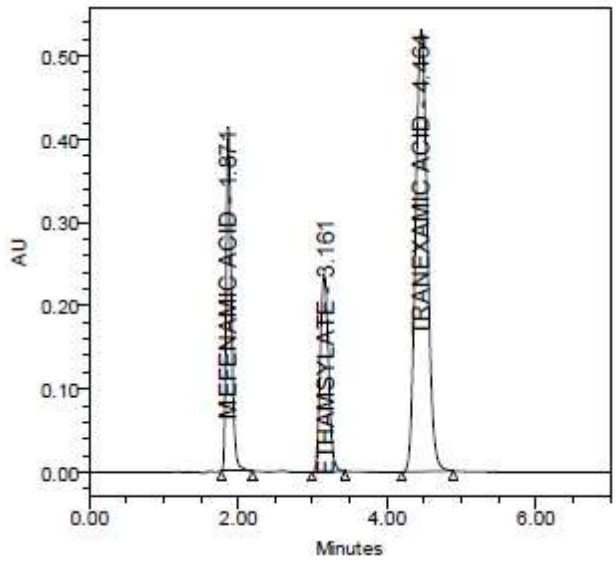


**Figure 7: Chromatogram of mefenamic acid, ethamsylate and tranexamic acid at 50% level**

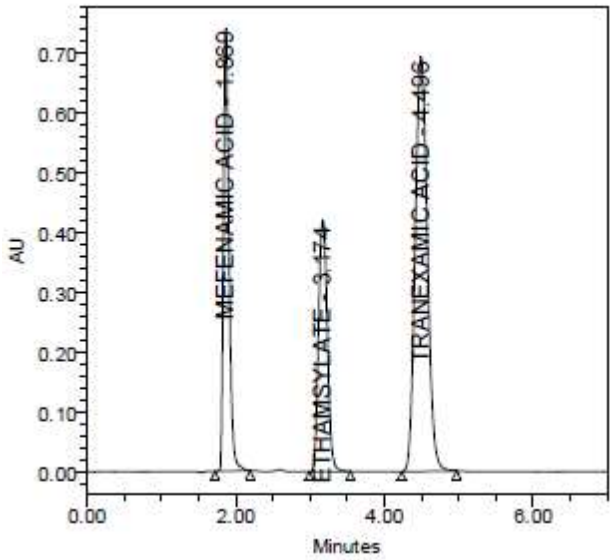


**Figure 8: Chromatogram of mefenamic acid, ethamsylate and tranexamic acid at 100% level**

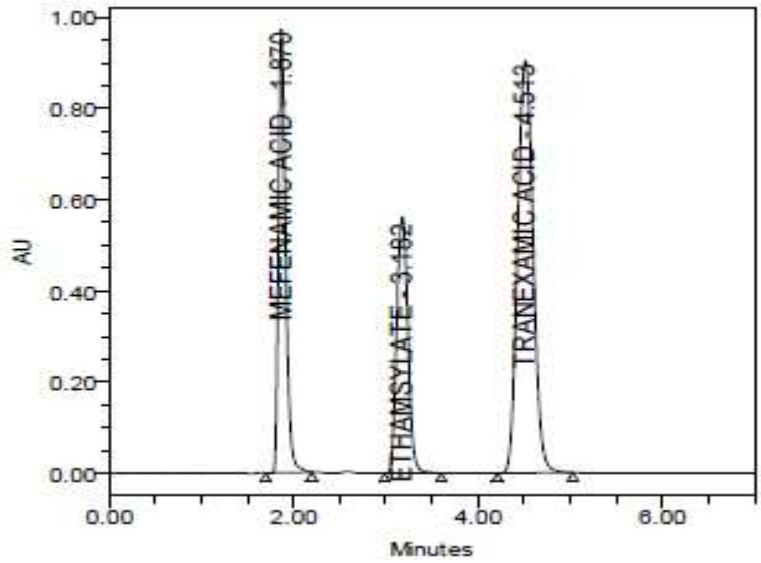


**Figure 9: Chromatogram of mefenamic acid, ethamsylate and tranexamic acid at 150% level**

The precision of the method was demonstrated by inter-day and intra-day variation studies. The results of the precision studies are tabulated in the Table 6. From the results obtained, the developed method was found to be precise for the simultaneous determination of mefenamic acid, ethamsylate and tranexamic acid.

**Table 6: Precision of the method**

| | Mefenamic acid | | Ethamsylate | | Tranexamic acid | |
|---|---|---|---|---|---|---|
| **Sample Wt (mg)** | **Area** | **%Assay** | **Area** | **%Assay** | **Area** | **%Assay** |
| 1322 | 3874997 | 99 | 3460943 | 100 | 7832572 | 99 |
| 1322 | 3878231 | 100 | 3468880 | 100 | 7834724 | 99 |
| 1322 | 3879903 | 100 | 3460821 | 100 | 7831496 | 99 |
| 1322 | 3871830 | 99 | 3467032 | 100 | 7830660 | 99 |
| 1322 | 3877364 | 100 | 3464954 | 100 | 7832996 | 99 |
| 1322 | 3879578 | 100 | 3464371 | 100 | 7830635 | 99 |

**Robustness**

Robustness of the method was determined by making slight changes in the chromatographic conditions such as column temperature and mobile phase flow rate. It was observed that there were no marked changes in the analytical performance of the method. The results are shown in Table 7. The results demonstrated that the proposed method is robust.

**Table 7: Robustness of the method**

| Sample no. | Sample Name | Retention time | Peak area | Theoretical plates | USP Tailing |
|---|---|---|---|---|---|
| **Mefenamic acid** | | | | | |
| 1 | Temp-1 | 1.859 | 3882298 | 4490 | 1.28 |
| 2 | Temp-2 | 1.843 | 3850868 | 3662 | 1.30 |
| 3 | Flow-1 | 2.341 | 4884979 | 4595 | 1.29 |

| | | | | | |
|---|---|---|---|---|---|
| 4 | Flow-2 | 1.559 | 3209370 | 3149 | 1.26 |
| **Ethamsylate** | | | | | |
| 1 | Temp-1 | 3.162 | 3450206 | 4012 | 1.09 |
| 2 | Temp-2 | 3.117 | 3434137 | 3209 | 1.10 |
| 3 | Flow-1 | 3.987 | 4355294 | 4214 | 1.06 |
| 4 | Flow-2 | 2.610 | 2857594 | 3459 | 1.29 |
| **Tranexamic acid** | | | | | |
| 1 | Temp-1 | 4.407 | 7840019 | 4978 | 1.09 |
| 2 | Temp-2 | 4.275 | 7771261 | 3303 | 1.09 |
| 3 | Flow-1 | 5.554 | 9854765 | 4108 | 1.07 |
| 4 | Flow-2 | 3.667 | 6471548 | 3494 | 1.22 |

**Limit of quantification and limit of detection**

Limit of quantification (LOQ) and limit of detection (LOD) gives information about the sensitivity of the method. The LOD and LOQ values for the mefenamic acid, ethamsylate and tranexamic acid are presented in Table 8. The chromatograms of LOD and LOQ are shown in Figures 10 and 11, respectively. The results indicated that the proposed method possess sufficient sensitivity.

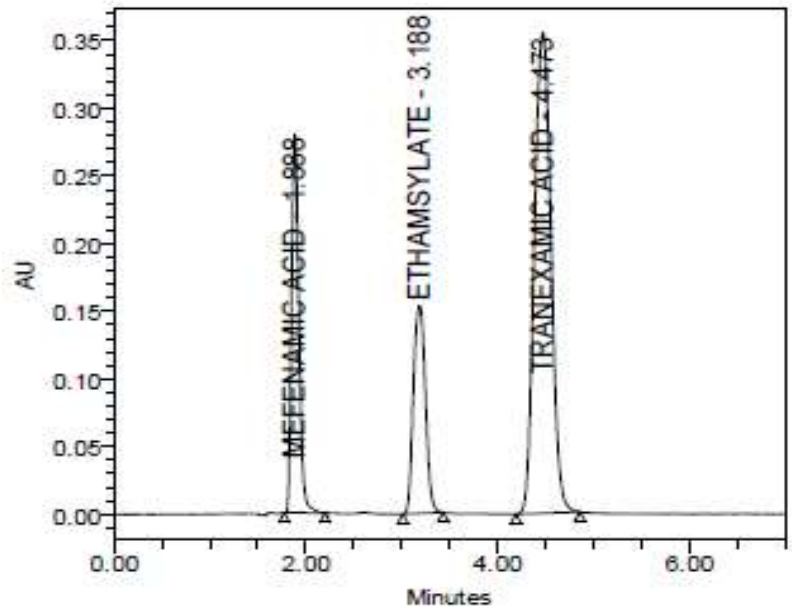


**Figure 10: Chromatogram of LOD**

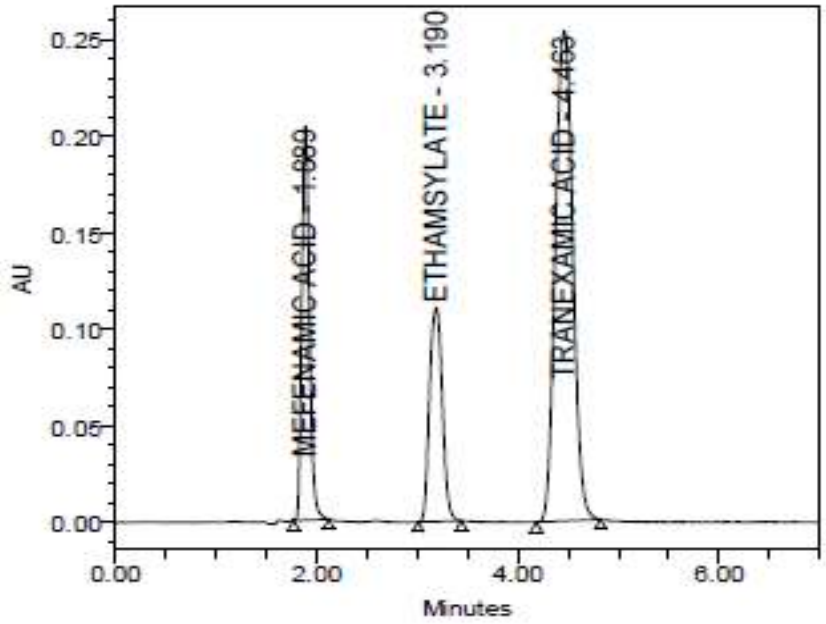


**Figure 11: Chromatograms of LOQ**

**Table 8: LOD and LOQ for mefenamic acid, ethamsylate and tranexamic acid**

| Sample Type | Sample name | RT | Area | Value |
|---|---|---|---|---|
| LOD | Mefenamic acid | 1.888 | 1493102 | 0.158 |
| LOQ | Mefenamic acid | 1.889 | 1085696 | 0.527 |
| LOD | Ethamsylate | 3.188 | 1336087 | 0.2183 |
| LOQ | Ethamsylate | 3.190 | 975004 | 0.7278 |
| LOD | Tranexamic acid | 4.473 | 4279170 | 0.321 |
| LOQ | Tranexamic acid | 4.463 | 3058976 | 1.071 |

## CONCLUSION

A HPLC with UV detection method was developed and validated for the simultaneous determination of mefenamic acid, ethamsylate and tranexamic acid in combined tablet dosage forms. The developed method was found to be simple, precise, accurate and sensitive for the simultaneous estimation. The method can easily and conveniently adopt for routine quality control analysis of mefenamic acid, ethamsylate and tranexamic acid in pure and in its pharmaceutical dosage forms.

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