Review Article

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New analytical method development and validation for the simultaneous estimation of tranexamic acid and mefenamic acid in pharmaceutical dosage forms

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ABSTRACT

A selective and sensitive reverse phase high performance liquid chromatography (RP-HPLC) has been developed for the separation and quantification of Tranexamic acid and Mefenamic acid in tablet dosage form and validated .The determination was carried out using Thermosil C18 column (25 cm ×4.6 mm id) as a stationary phase and mobile phase comprised of Methanol: Sodium acetate buffer in proportion of 65:35(v/v) with pH adjusted to 3±0.5 by using orthophosphoricacid. The flow rate was 1.0ml/min and the eluent was monitored at 256nm.The retention time of Tranexamic acid and Mefenamic acid were 2.45 ±0.028 min and 4.31±0.018 min respectively.. The Coefficient of correlation and percentage recoveries of Tranexamic acid and Mefenamic acid were 0.9986 and 100.0l % and 0.9994 and 99.98% respectively. The method is validated for accuracy, Precision, ruggedness and Robustness. The proposed method is successfully applied for the simultaneous determination of both drugs in commercial tablet preparation. The results of the analysis have been validated statistically and by recovery studies.

Keywords: Tranexamic acid Mefenamic acid, Simultaneous estimation, RP-HPLC method.

INTRODUCTION

Tranexamic acid ((figure1) is chemically trans-4-aminomethyl-cyclohexaecarboxylic acid [1]. It competitively inhibits activation of plasminogen, there by reducing conversion of plasminogen to plasmin, an enzyme that degrades fibrin clots, fibrinogen, & other plasma protein, including the procoagulant facture V & VIII. It is used for controlling abnormal bleeding in a number of diseases. Tranexamic acid is official in British Pharmacopeia [2]. Mefenamic acid, chemically N-[(2, 3-dimethyl phenyl) amino] benzoic acid **(**Figure 1**),** official in USP, BP and IP [3] is a potent non-steroidal anti-inflammatory drug with analgesic and antipyretic properties. It shows preferential inhibition of cyclooxygenase-2 and there by inhibits the prostaglandin synthesis [4]. It is used in the treatment of osteoarthritis, nonarticular rheumatism, healing of wounds, sport

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injuries, antichloristic, rheumatoid arthritis and other painful musculoskeletal illnesses [5]. Its oral bioavailability is very low due to poor solubility in water and insufficient dissolution rate. New tablet formulation in combination of tranexamic acid 500 mg and mefenamic acid 250 mg is commercially available in Indian market for treatment of menorrhagia during menstruation. Literature survey revealed that few analytical methods have been reported for individual estimation of tranexamic acid and mefenamic acid. [6-17]. The present study describes a precise, accurate, specific and sensitive RP-HPLC method as per ICH guidelines for the simultaneous estimation of Tranexamic acid and Mefenamic acid in tablets as well as for application to dissolution testing of tablet formulations [18].

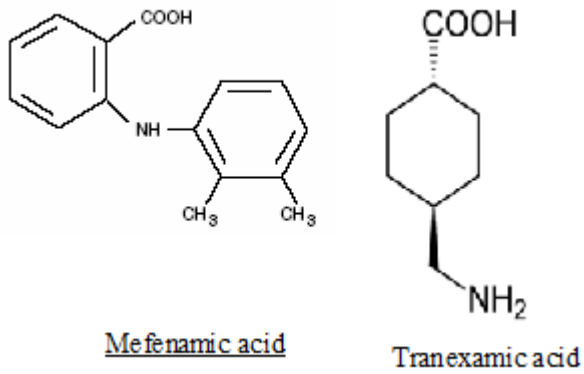

Figure1.Structure of Tranexamic acid and Mefenamic acid.

EXPERIMENTATION

Equipment

Chromatographic separation was performed on Waters HPLC system consist of model 2695 having PDA detector and Rheodyne injector with 20µl loop volume. Waters Empower software was applied for data collecting and processing.

Reagents and chemicals

Acetonitrile and water of HPLC grade were procured from Rankem lab ltd. working standard of tranexamic acid was provided by Orchid pharmaceuticals Chennai and Mefenamic acid was provided by Emcure Pharmaceutical Ltd, Pune. Sodium acetate and orthophosphoricacid were A.R grade from Merck chemicals Mumbai, India. Tablets two different brands were purchased from Indian market, containing 500 mg of tranexamic acid and 250 mg of mefenamic acid per tablet

Optimized chromatographic Condition

A Thermosil C18 column (25cm×4.6mm, 5µ) column was used as the stationary phase. A mixture of Methanol: Sodium acetate buffer in proportion of 65:35(v/v) was used as a mobile phase and P^H 3.0 adjusted with orthophosphoric acid. It was filtered through 0.45µ membrane filter and degassed. The mobile phase was pumped at 1 ml/min. The eluents were monitored at 238nm.The injection volumes of samples and standard were 20µl.

STANDARD PREPARATION

Preparation of standard solution

10 mg of Tranexamic acid and 10mg of Mefenamic acid 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) . The working standard solutions were prepared and further diluted in mobile phase to Tranexamic acid and Mefenamic acid contain a mixture of in over the linearity ranges from240-640 µg/ml and 120-320 µg/ml.

Sample preparation

Twenty tablets were weighed and finely powdered. A quantity of powder equivalent to5 mg of Mefenamic acid and 10mg of Tranexamic acid was weighed and transferred to a 25 ml volumetric standard flask and added 10 ml of mobile phase. The sample was kept in an ultrasonic bath for 20 min and further diluted to 25 ml by using mobile phase to get 200µg/ml of Mefenamic acid and 400µg/ml of Tranexamic acid. Then it is filtered through 0.22µ membrane filter paper.20µl of this solution was injected in to HPLC system and chromatograms were recorded. Concentrations of Tranexamic acid and Mefenamic acid in the tablet formulation were calculated by comparing area of the sample with that of standard. The percentage assay of individual drug was calculated and presented in Table1.

Table1: Table for Assay

Tablet formulation	Drug	Amount present mg	Amount found* (mg/tab)	% label claim*
T1	Tranexamic acid	500	499.98	99.99
	Mefenamic acid	250	248.97	99.58
T2	Tranexamic acid	500	498.71	99.74
	Mefenamic acid	250	249.97	99.98

T1 and T2 are two different brands of tablet formulations.*Each value is average of six determinations.

RESULTS AND DISCUSSION

The proposed HPLC method required fewer reagents and materials and it is simple and less time consuming. This method could be used in quality control test in Pharmaceutical industries. The chromatograms sample and standard solution of Tranexamic acid and Mefenamic acid were shown in (Figure.1) and (Figure.2). There was clear resolution between Tranexamic acid and Mefenamic acid with retention time of 2.457and4.308 minutes respectively.

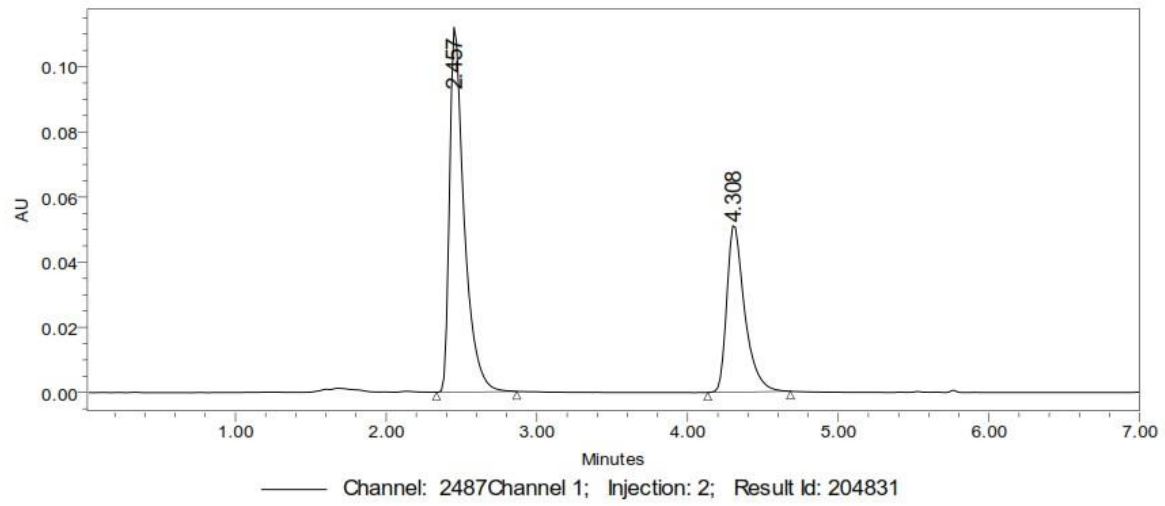

Figure 1: Typical Chromatogram of standard solution of Tranexamic acid and Mefenamic acid.

VALIDATION OF THE METHOD

System suitability

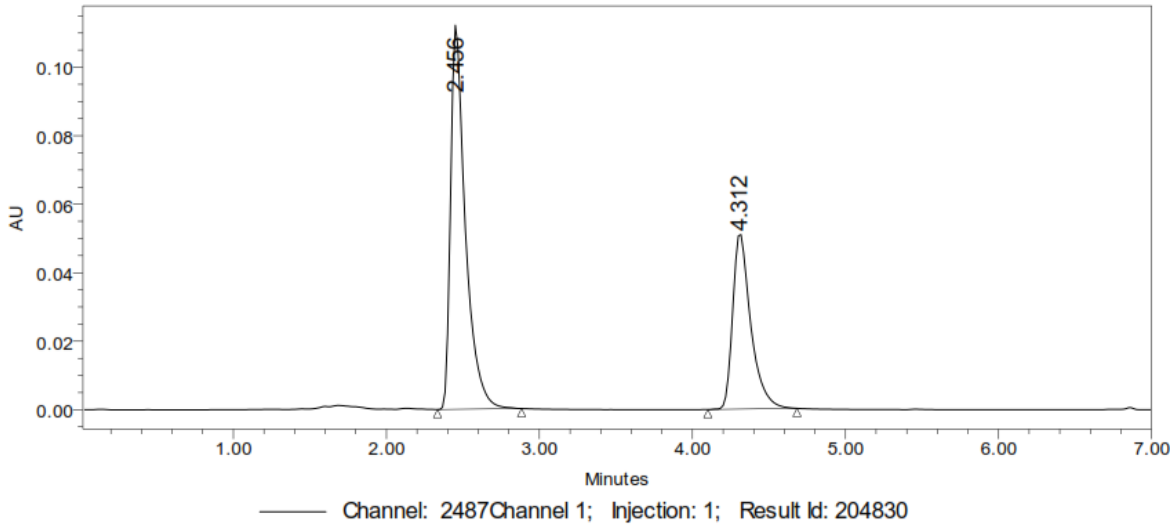

Figure 2: Typical Chromatogram of sample solution of Tranexamic acid and Mefenamic acid

The column efficiency, resolution and peak symmetry were calculated for the standard solutions (Table.2). The values obtained demonstrated the suitability of the system for the analysis of this drug combination and the system suitability parameters fall with in±3% standard deviation range during performance of the method. Here tailing factor for peaks of Tranexamic acid and Mefenamic acid was less than 2% and resolution was satisfactory. The peaks obtained for Tranexamic acid and Mefenamic acid were sharp and have clear base line separation.

TableNo2: System Suitability

S.No	Parameters	Tranexamic acid	Mefenamic acid
1	Capacity factor	1	1
2	Theoretical plate	4233	5695
3	Asymmetry of the peak	1.2	0.69
4	Retention time (min)	2.451	4.314
5	Resolution	5.4	

Linearity

The response for the detector was determined to be linear over the range of 120-320µg/ml (120,160,200,240,280,320) of Tranexamic acid and 240-640µg/ml (240,320,400,480,560,640) for Mefenamic acid. Each of this concentration was injected in six times to get reproducible response. The calibration curve was plotted as concentration of the respective drug versus the response at each level. The proposed method was evaluated by its correlation coefficient and intercept value calculated in the statistical study. The results show that an excellent correlation exits between response factor and concentration of drugs within the concentration range indicated above. (Table 3)

Table3: Summary of analytical method validation

S.No	Parameters	Acceptance criteria	Tranexamic acid	Mefenamic acid
1	Linearity	r^2=0.995 to1.0	0.9996	0.9992
2	Specificity	No interference with placebo	specific	specific
3	Accuracy(Recovery studies)	Recovery98.0-102.0%	99.98%	101.02%
4	**Precision**			
	Intraday	RSD NMT 2.0%	0.1052	0.4622
	Interday	RSD NMT 2.0%	0.2526	0.2723
5	**Robustness**			
	Change inflow rate	NMT±1%	0.3%	0.4%
	Change in mobile phase ratio	NMT±1%	0.2%	0.4
	Change in p^H	NMT±1%	0.2%	0.3%
6	Limit of detection µg/ml	----------	0.5µg/ml	1µg/ml
	Limit of Quantification µg/ml	----------	1.5µg/ml	3µg/ml

Precision and Accuracy

Recovery studies were carried out by applying the standard addition method. A known amount of standard Tranexamic acid and Mefenamic acid corresponding to 80%, 100%, and 120% of the label claim was added to pre analyze sample of tablet dosage form separately. The recovery studies were carried out six times at each level of recovery. From the data obtained, recoveries of standard drugs were found to be accurate (Table.3.). The %RSD of interday and intraday precision obtained was less than2%for both the drugs. The intraday and interday precision of Tranexamic acid was 0.1052 and 0.2526 and Mefenamic acid was 0.4622and 0.2723 respectively. From the data obtained, the developed HPLC method was found to be precise and accurate.

Specificity of the method

The specificity of the method was checked for the interference of impurities in the analysis of a blank solution (without any sample) and then a drug solution of 20 µg/ml was injected into the column, under optimized chromatographic conditions, to demonstrate the separation of both Tranexamic acid and Mefenamic acid from any of the impurities, if present. As there was no interference of impurities and also no change in the retention time, the method was found to be specific and also confirmed with the results of analysis of formulation.

LOD and LOQ

Limit of detection (LOD) and limit of quantification (LOQ) were calculated as 3.3 ∂/S and 10 ∂/S, respectively as per ICH guidelines, where ∂ is the standard deviation of the response (*y*-intercept) and *S* is the slope of the calibration plot. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3).The LOD for Tranexamic acid and Mefenamic acid was found to be 0.51µg/ml and 1.0µg/ml, respectively. The LOQ is the smallest concentration of the analyte which gives response that can be accurately quantified (signal to noise ratio of 10).The LOQ was 1.52µg/ml and 3µg/ml for Tranexamic acid and Mefenamic acid respectively. (Table3)

Ruggedness and Robustness

The ruggedness of the method was determined by carrying out the experiment on different instrument like Waters HPLC and Agilent HPLC by different operators using different columns of similar type like HypersilC18, ZorbaxC18 column. Robustness of the method was determined by making slight changes in the experimental conditions such as the composition of the mobile phase, pH of the mobile phase, and flow rate of the mobile phase and the chromatographic characteristics were evaluated. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed, are rugged and robust.

CONCLUSION

The proposed RP-HPLC method for the simultaneous estimation of Tranexamic acid and Mefenamic acid in combined dosage forms is accurate, precise, linear, rugged, robust, simple and rapid. Hence the present RP-HPLC method is suitable for the quality control of the raw materials, formulations and dissolutions studies.

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