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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF TRANEXAMIC ACID AND MEFENAMIC ACID IN TABLET DOSAGE FORM

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ABSTRACT
To develop simple, accurate, precise, rapid and economical Stability Indicating RP-HPLC method for the Tranexamic acid and Mefenamic acid in dosage form method included Shimadzu LC-2010, using Hypersil BDS-C18 (250 * 4.6 mm, 5µm) column and with mobile phase composition of Phosphate Buffer: Acetonitrile (70:30 % v/v)pH 6, at a flow rate of 1ml/min was used. Detection was carried out at 215 nm. Retention time of Tranexamic acid and Mefenamic acid was found to be 5.277 min and 3.583 min. For Stability Study Drug Were Subjected to acid hydrolysis, alkaline hydrolysis, oxidative degradation and thermal degradation. The linear of the Proposed method was investigated in the range of 10-30 µg/ml and 5-15 µg/ml for Tranexamic acid and Mefenamic acid. The limit of detection were0.704 µg/ml and 0.908 µg/ml of Tranexamic acid and Mefenamic acid and the limit of quantification were 2.156 µg/ml and 2.754 µg/ml of Tranexamic acid and Mefenamic acid.

KEYWORDS: Tranexamic acid, Mefenamic acid RP-HPLC method, forced degradation, validation.

MATERIALS AND METHODS
Tranexamic acid Trans (aminomethyl) cyclohexane carboxylic acid it act as Anti fibrinolytic agent it is official in British pharmacopeia 2014 and Indian pharmacopeia 2014 it is Freely soluble in water and glacial acetic acid Molecular weight of Tranexamic acid 157.21 is gm/mol and formula is $C_8H_{15}NO_2$.

Mefenamic acid 2-(2,3-dimethylanilino) benzoic acid it act as Analgesic, anti-pyretic and anti- inflammatory it is official in British pharmacopeia 2014 and Indian pharmacopeia 2014 it is Freely soluble in 0.1N NaOH acid Molecular weight of Mefenamic acid 241.2851 is gm/mol and formula is $C_{15}H_{15}NO_2$.

Tranexamic acid and Mefenamic acid is obtained from Macleods Pharmaceutical Ltd Kachigam Daman.

Instrumentation and Chromatographic method
The analysis of drug was carried on RP- HPLC, by using Shimadzu LC-2010, using Hypersil BDS- C_{18} (250 * 4.6 mm, 5µm) column and with mobile phase composition of Phosphate Buffer: Acetonitrile (70:30 % v/v)pH 6, at a flow rate of 1ml/min was used. Detection was carried out at 215 nm. Retention time of Tranexamic acid and Mefenamic acid was found to be 5.277 min and 3.583 min.

Determination of maximum absorbance
The standard solution of Tranexamic acid and Mefenamic acid were scanned in range 200-400nm against mobile phase as blank Isobestic point of Tranexamic acid and Mefenamic acid 215nm Thus the wavelength selected for the determination of Tranexamic acid and Mefenamic acid 215nm.

Preparation of stock and standard solutions
Accurately weighed 20mg of Tranexamic acid and 10mg of Mefenamic acid were dissolved in 100 ml volumetric flask containing 100 ml of Methanol which is considered as stock solution. Working standard solution of Tranexamic acid and Mefenamic acid were prepared by making various dilutions of the drug solution from the stock solution. Five sets of the drug solution were prepared in the mobile phase containing 10-30µg/ml of Tranexamic acid and 5-15 µg/ml of Mefenamic acid. Each of this drug solution was injected into the column and the peak area and retention time was recorded.

Assay of Marketed formulation (Branded name of tablet Trenaxa-MF

Twenty tablets were weighed and average weight of a single tablet was calculated. Tablets were crushed and mixed using a mortar and pestle. Then drug sample equivalent to 20 mg of Tranexamic acid and 10mg of Mefenamic acid were accurately weighed and transferred into a 100ml volumetric flask and mixed with known amount of methanol and the active pharmaceutical ingredients were extracted into the methanol followed by ultra- sonication and then filtered through a nylon membrane of pore size 0.45µm. The drug sample was diluted by adding methanol to obtain a stock solution of 200µg/ml of Tranexamic acid and 100 µg/ml of Mefenamic acid.

Method validation

The Proposed method was validated according to ICH guidelines. The parameters assessed were linearity, precision, accuracy, LOD and LOQ.

System Suitability: System suitability tests are an integral part of liquid chromatography. They are used to verify that resolution and reproducibility of chromatography system are adequate for the analysis to be done. System Suitability was performed on standard solution and system suitability parameters were calculated at the start of study for each parameter.

Linearity and Range: The linearity was determined at Three levels over the range of 10-30µg/ml Tranexamic acid and 5-15µg/ml Mefenamic acid. Peak area of above linearity solution preparations were taken at each concentration three times.

Accuracy: Recovery studies were carried out by addition of standard drug to the sample at 3 different concentration levels (80%, 100% and 120%) taking into consideration percentage purity of added bulk drug samples. These solutions were subjected to re-analysis by the proposed method and Results are calculated.

Precision Repeatability Study: Standard solutions of 10,20,30 µg/ml Tranexamic acid and 5,10,15 µg/ml Mefenamic acid were prepared and chromatograms were recorded. Area was measured of the same concentration solution three times and %RSD was calculated.

Intra-day precision

Mixed solutions containing 10,20,30 µg/ml Tranexamic acid and 5,10,15 µg/ml Mefenamic acid were analysed three times on the same day % R.S.D was calculated.

Inter-day precision

Mixed solutions containing 10,20,30 µg/ml Tranexamic acid and 5,10,15 µg/ml Mefenamic acid were analysed on three different days and % R.S.D was calculated.

Limit of Detection and Limits of Quantitation Limit of Detection (LOD): From the linearity curve equation, the standard deviation (SD) of the intercepts (response) was calculated. The limit of detection (LOD) of the drug was calculated by using the following equation designated by International Conference on Harmonization (ICH) guideline

LOD = 3.3× Intercept / Slope

Limit of Quantitation (LOQ)

The limit of quantitation (LOQ) of the drug was calculated by using the following equation designated by International Conference on Harmonization (ICH) guideline: LOQ = 10 × Intercept / Slope

Robustness

The robustness of the method was established by making deliberate minor variations in the following method parameters

a) pH of mobile phase: ± 0.2

b) Flow rate : ± 0.2 ml/min

c) Change in the ratio of component in the mobile phase: ± 2%.

Stability studies

Stability Studies was carried out on the drug in order to check the stability of the drug by providing various stress conditions like acid, base, oxidation and thermal degradation compared with normal conditions. The purpose of force degradation method is to provide evidence that the analytical method is efficient in determination of drug substances in commercial drug product in the presence of its degradation products.

Acidic hydrolysis: Take 1 ml solution of Tranexamic acid 200 µg/ml and Mefenamic acid 100 µg/ml 2 ml of 0.1N HCl was added. The solution was heated for 4 hr and transferred to a 10ml volumetric flask, cooled, neutralized by 0.1N NaOH and diluted up to mark with methanol to get final concentration 200 µg/ml of Tranexamic acid and 100 µg/ml of Mefenamic acid

Alkaline hydrolysis: Take 1 ml solution of Tranexamic acid 200 µg/ml and Mefenamic acid 100 µg/ml 2 ml of 0.1N NaOH was added. The solution was heated for 4 hr and transferred to a 10ml volumetric flask, cooled, neutralized by 0.1N HCl and diluted up to mark with methanol to get final concentration 200 µg/ml of Tranexamic acid and 100 µg/ml of Mefenamic acid.

Oxidative degradation: Take 1 ml solution of Tranexamic acid 200 µg/ml and Mefenamic acid 100 µg/ml 2 ml 3% H_2O_2 was added at room temperature for 4 hours and transferred to a 10ml volumetric flask, cooled diluted up to mark with methanol to get final concentration 200 µg/ml of Tranexamic acid and 100 µg/ml of Mefenamic acid.

Thermal degradation: Take 1 ml solution of Tranexamic acid 200 µg/ml and Mefenamic acid 100 µg/ml heat the solution for 4 hr at 105^{0}C and transferred to a 10ml volumetric flask, cooled diluted up to mark

with methanol to get final concentration 200 µg/ml of Tranexamic acid 100 µg/ml of Mefenamic acid.

RESULT AND DISCUSSION

Method development

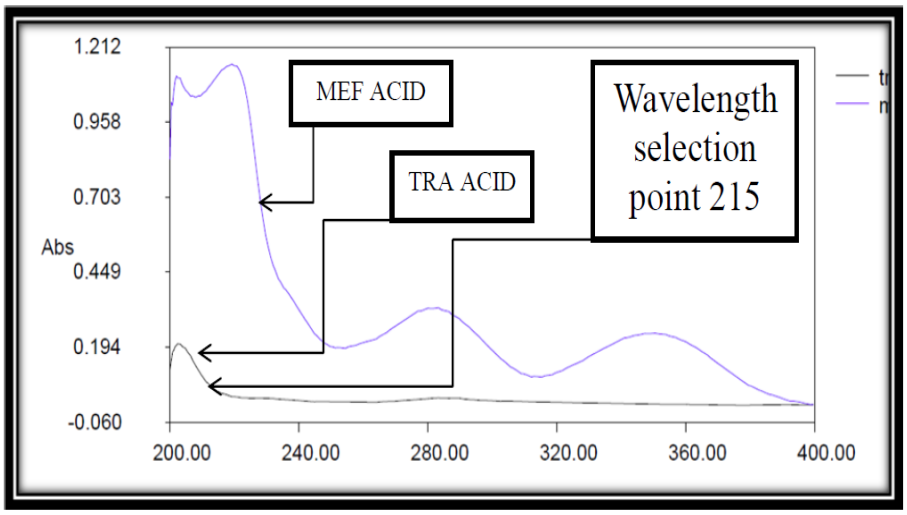

Figure 1 Determination of detection wavelength

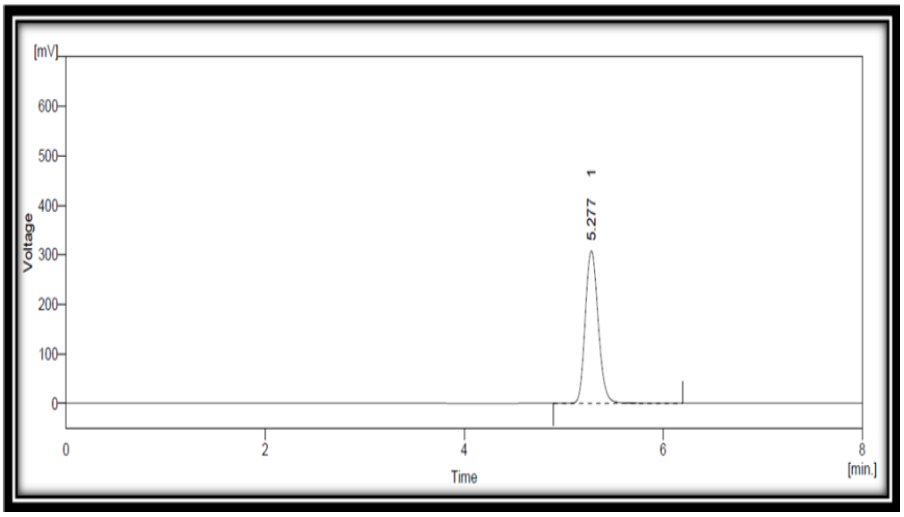

Figure no:- 2 Chromatogram of Tranexamic acid

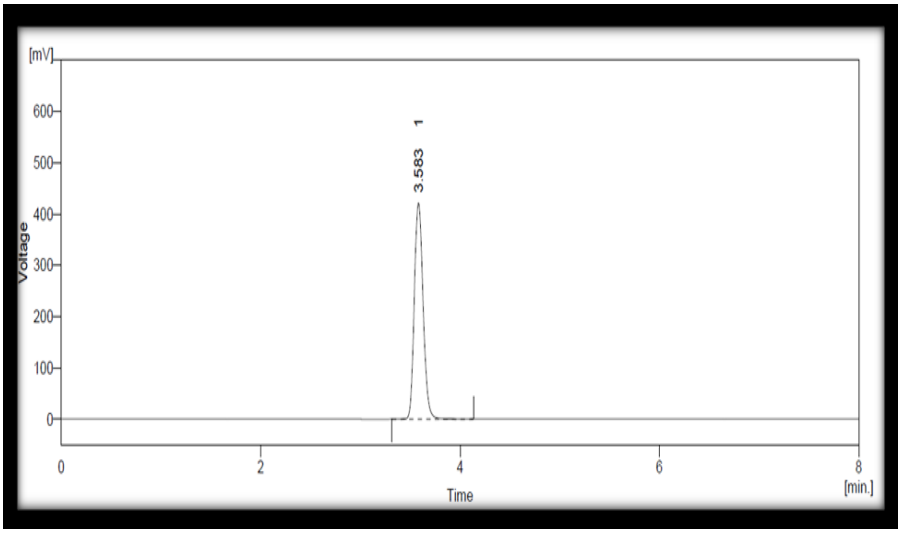

Figure no:- 3 Chromatogram of Mefenamic acid

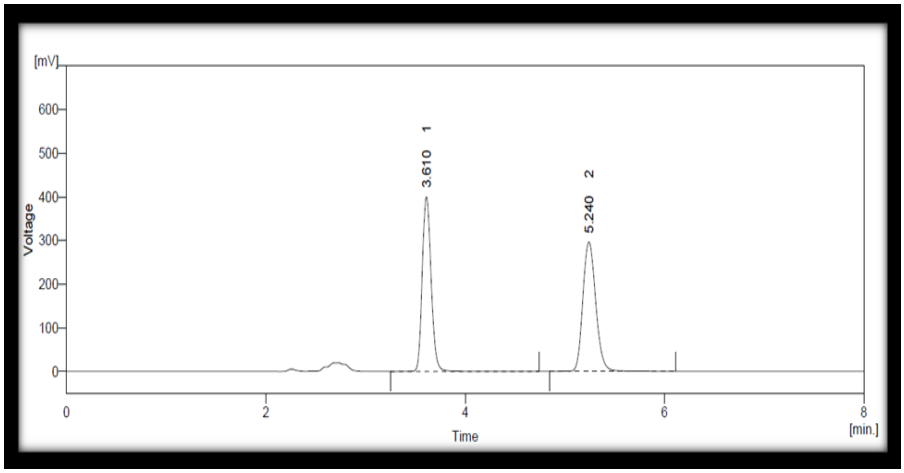

Figure no:- 4 Chromatogram of formulation

Linearity

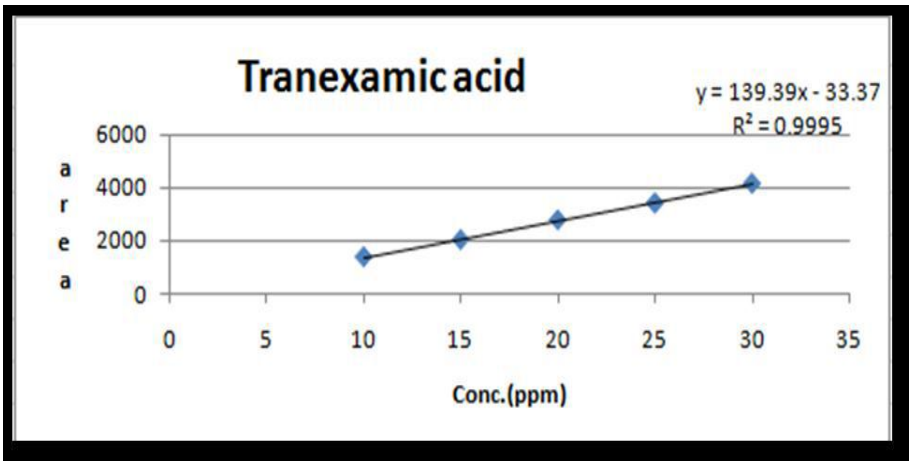

Figure no:- 5 Calibration curve of Tranexamic acid

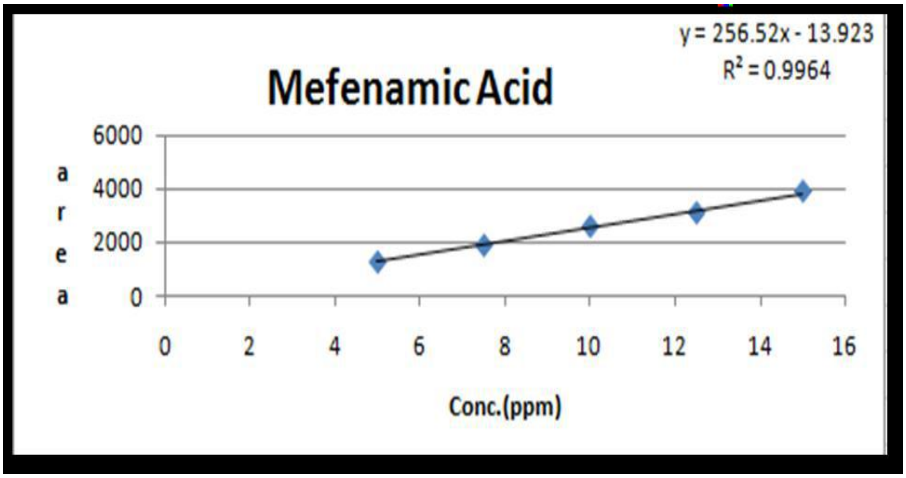

Figure no:- 6 Calibration curve of Mefenamic acid

Table 1 System suitability parameters

Parameters	Data obtained		Standard value
	MEAN± S.D. (n=6) Tranexamic acid	MEAN± S.D. (n=6) Mefenamic acid	
Theoretical plates	7470 ± 3.033	7153 ± 2	Not less than 2000
Tailing factor	1.228 ± 0.003	1.208 ± 0.003	Not greater than 2.0
Retention time	5.26 ± 0.020	3.59 ± 0.041	-
Resolution	8.077	-	More than 1.5

Accuracy

Table 2 Recovery study of Tranexamic acid

Level	Actual Conc. (µg/ml)	Amount of standard spiked (µg/ml)	Total amount (µg/ml)	Peak Area of sample	% Recovery	Mean % Recovery ± S.D (n=3)	%RSD
80%	10	8	18	2413.18	98.84	99.80±0.839	0.8408
	10	8	18	2430.07	100.42		
	10	8	18	2427.11	100.14		
100%	10	10	20	2683.32	99.19	99.54±0.299	0.3005
	10	10	20	2690.57	99.73		
	10	10	20	2689.95	99.69		
120%	10	12	22	2968.75	100.37	99.68±0.604	0.6063
	10	12	22	2952.35	99.36		
	10	12	22	2951.45	99.30		

Table 2 Recovery study of Mefenamic acid

Level	Actual Conc. (µg/ml)	Amount of standard spiked (µg/ml)	Total amount (µg/ml)	Peak Area of sample	% Recovery	Mean % Recovery ± S.D (n=3)	%RSD
80%	5	4	9	2303.42	99.78	100.90±1.040	1.031
	5	4	9	2324.26	101.84		
	5	4	9	2316.44	101.07		
100%	5	5	10	255.99	99.80	100.48±0.725	0.721
	5	5	10	2574.24	101.24		
	5	5	10	2563.5	100.39		
120%	5	6	11	2829.12	101.17	100.36±0.712	0.710
	5	6	11	2812.39	100.07		
	5	6	11	2808.88	99.83		

Precision

Table 4 Repeatability study of both the drugs

Drug	Conc(µg/ml	Intraday precision		Intraday precision	
		Mean ± S.D (n=3)	%R.S.D	Mean± S.D (n=3)	%R.S.D
TRA	10	1360.15±16.05	1.180	1354.39±22.10	1.6311
	20	2751.93±25.74	0.935	2750.06±20.81	0.757
	30	4127.80±33.94	0.822	4121.04±33.04	0.801
MEF	5	1270.06±12.36	0.973	1264.62±18.35	1.451
	10	2569.23±27.82	1.089	2567.49±23.97	0.933
	15	3856.13±34.61	0.897	3856.30±24.08	0.624

Limit of Detection and Limits of Quantitation Limit of Detection (LOD)

Table 7 LOD and LOQ of both the drugs

Drugs	LOD (µg/ml)	LOQ (µg/ml)
Tranexamic acid	0.704	2.136
Mefenamic acid	0.908	2.754

Force Degradation studies

Table no 8 Force Degradation studies of both drug

Stress Condition	%Degradation of API		%Degradation of pharmaceutical dosage form	
Stress condition	Tranexamic acid	Mefenamic acid	Tranexamic acid	Mefenamic acid
Acid Hydrolysis	17.481	15.537	15.964	11.579
Alkaline Hydrolysis	13.677	13.581	12.235	10.134
Oxidative	10.091	11.708	10.437	10.477
Thermal	11.729	12.822	13.723	11.745

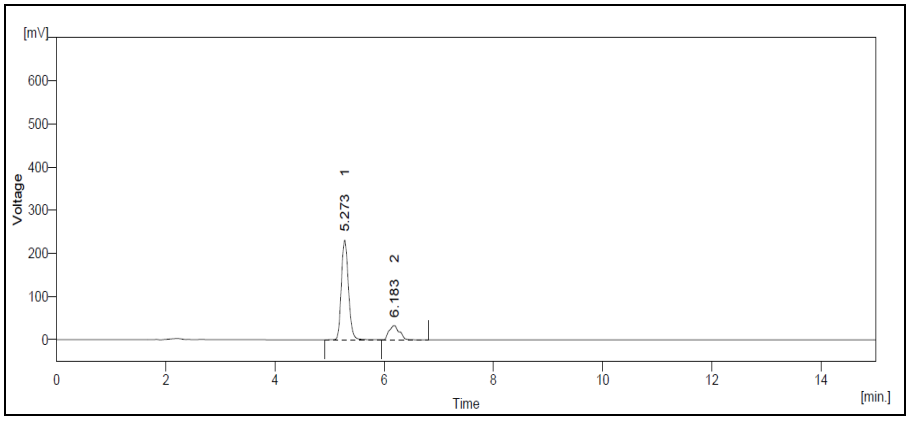

Figure no:- 7 Acid Hydrolysis of Tranexamic acid

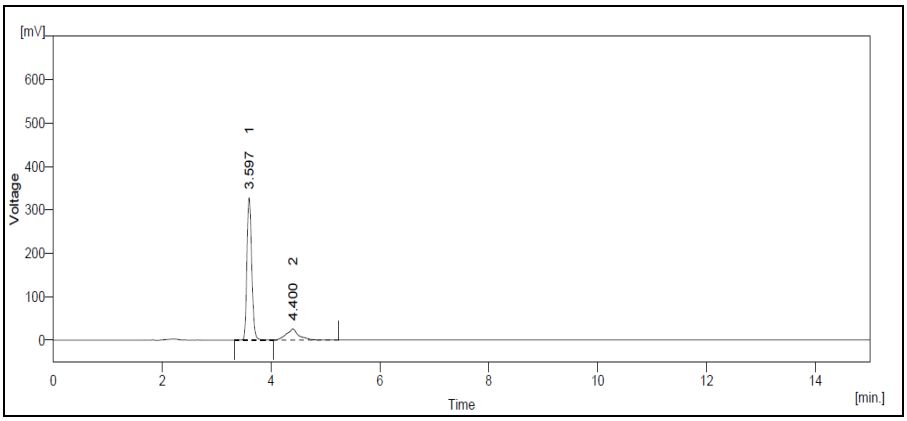

Figure no:- 8 Acid Hydrolysis of Mefenamic acid

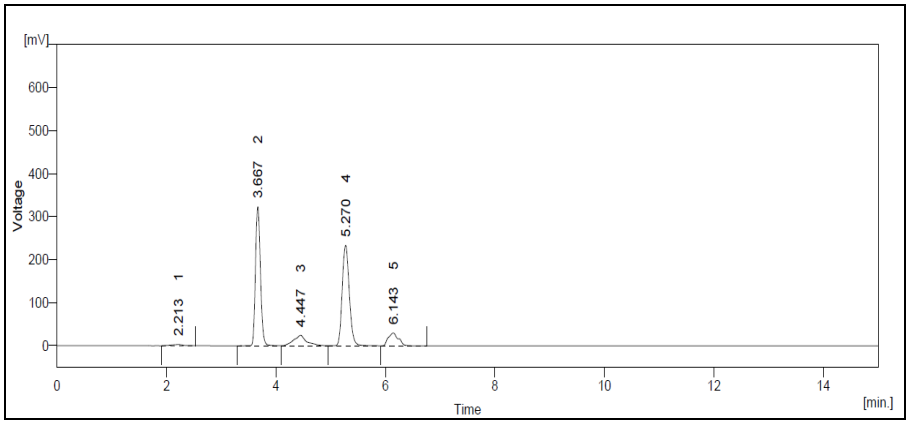

Figure no:- 9 Acid Hydrolysis of Formulation

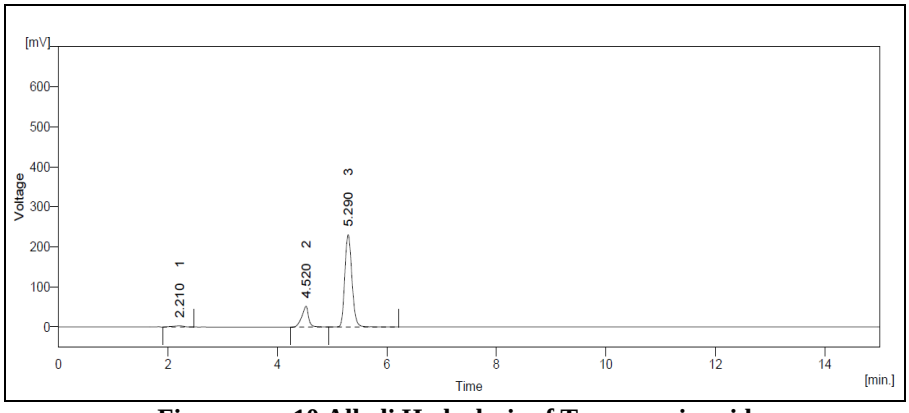

Figure no:- 10 Alkali Hydrolysis of Tranexamic acid

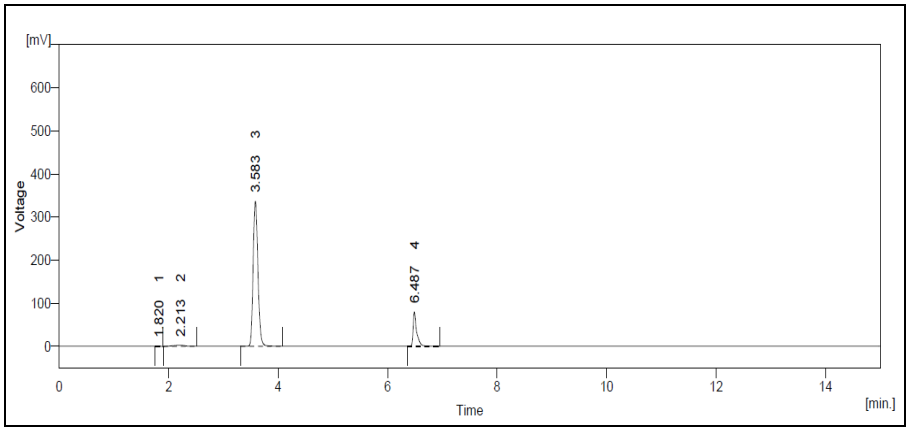

Figure no:- 11 Alkali Hydrolysis of Mefenamic acid

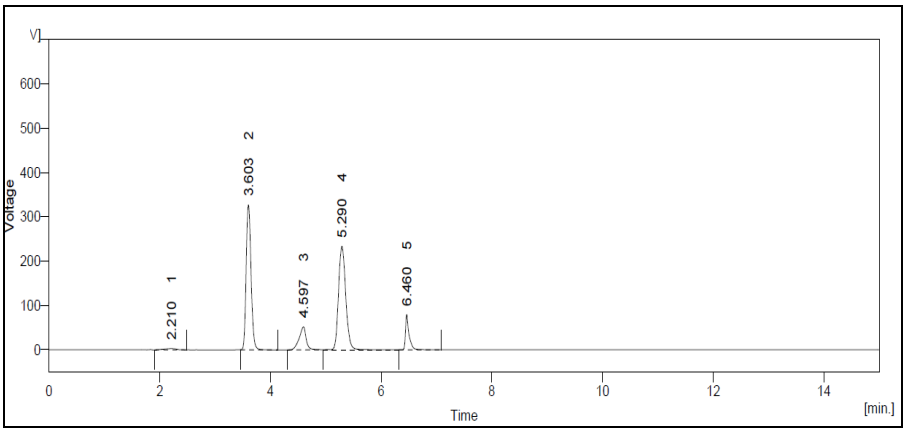

Figure no:- 12 Alkali Hydrolysi of Formulation

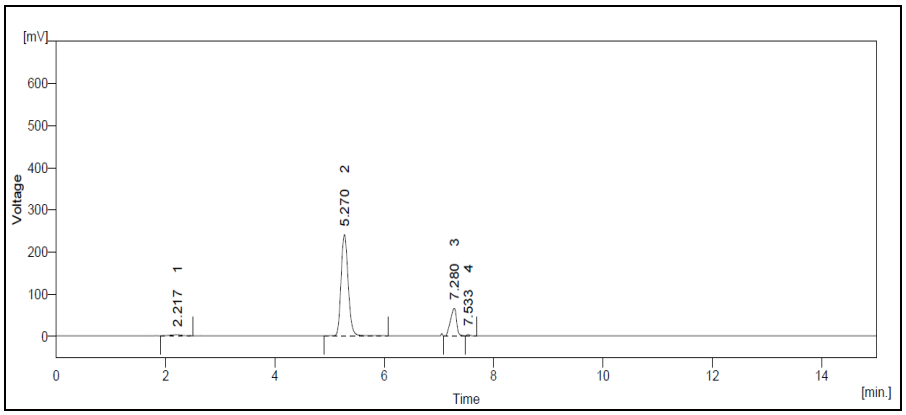

Figure no:- 13 Oxidative Hydrolysis of Tranexamic acid

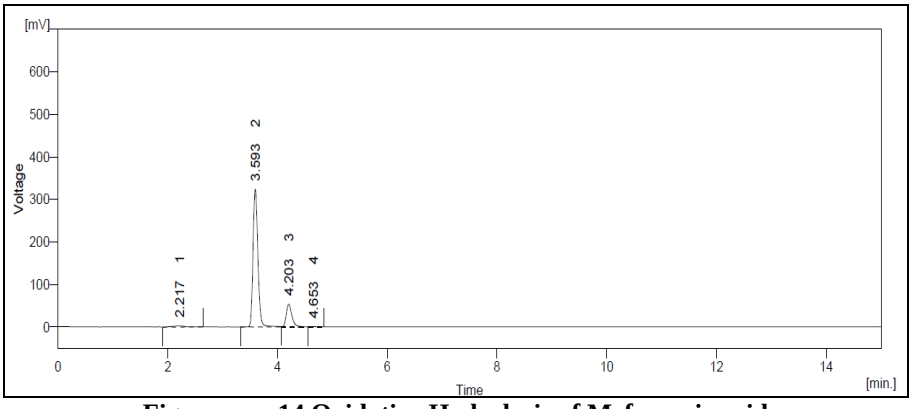

Figure no:- 14 Oxidative Hydrolysis of Mefenamic acid

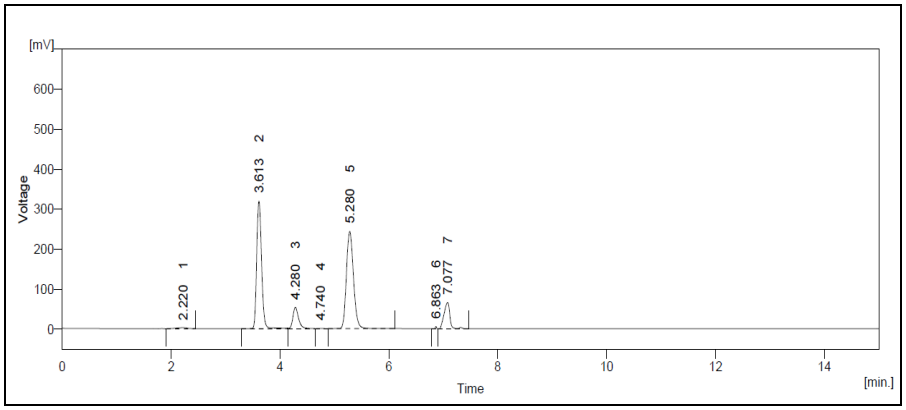

Figure no:- 15 Oxidative Hydrolyisis of Formulation

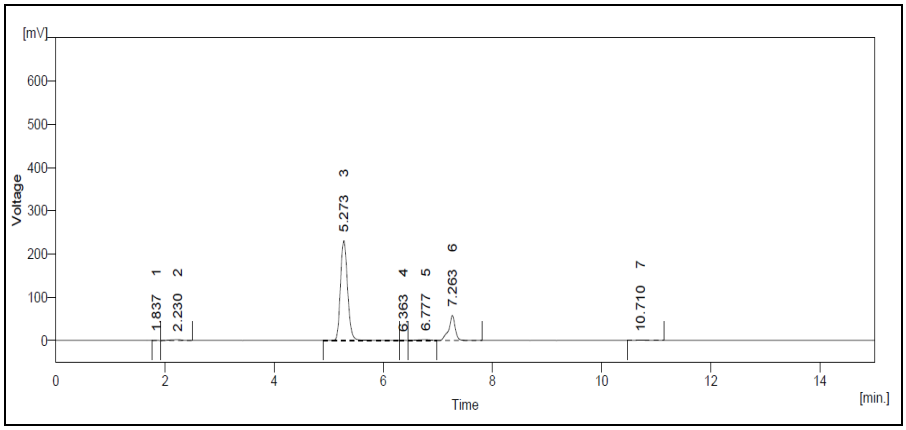

Figure no:- 16 Thermal Hydrolyisis of Tranexamic acid

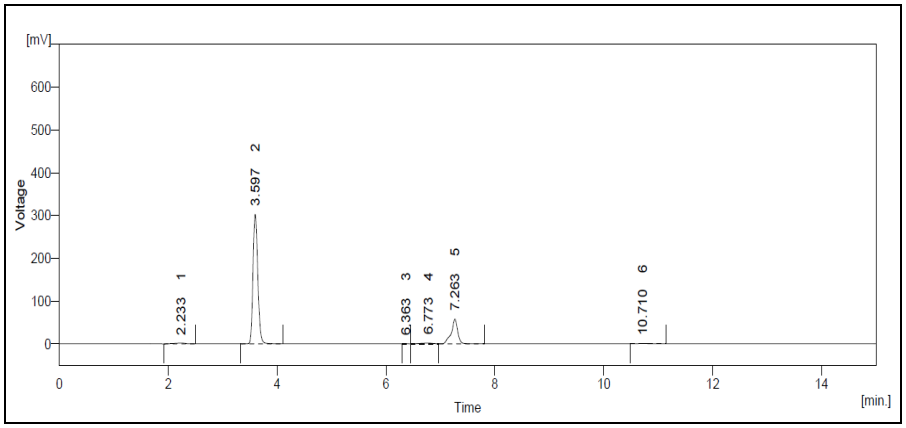

Figure no:- 17 Thermal Hydrolysis of Mefenamic acid

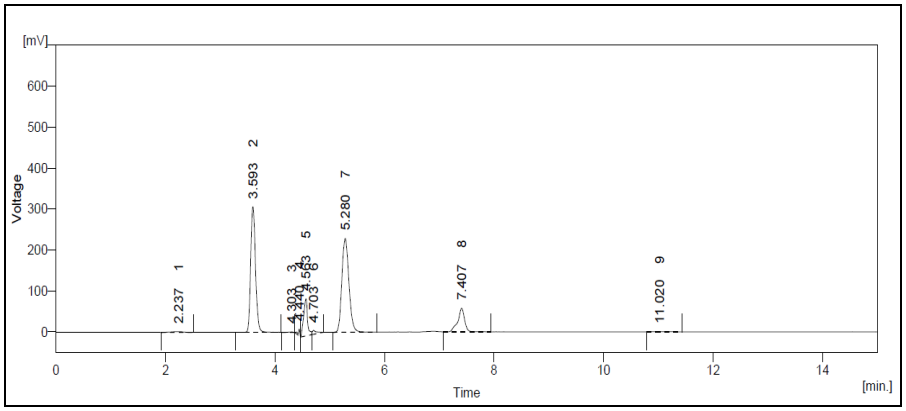

Figure no:- 18 Thermal Hydrolysis of Formulation

CONCLUSION

- Development HPLC Method can resolve all Degradant peak of both drug. No chromatographic interference from tablet excipients was found.
- It is concluded that the developed method is specific. The test parameters were also performed and were found to be within acceptable criteria. The method can be successfully employed for the simultaneous determination of Tranexamic acid and Mefenamic acid in pharmaceutical formulation.

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