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Development and Validation of Stability Indicating RP-HPLC Method for Determination of Mefenamic Acid in Suspension Dosage Form

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ABSTRACT

A Simple, fast, accurate, precise and selective stability-indicating reverse phase high performance liquid chromatographic method was developed and validated for determination of Mefenamic Acid in suspension dosage form. The chromatographic separation was done using C18 column (250x4.6mm, 5µ particle size) (Hypersil BDS) with mobile phase containing phosphate buffer pH 3.0: acetonitrile in the ratio of 20:80 % v/v at flow rate of 1.5 ml/min having injection volume 10µL and detection wavelength at 285 nm. The retention time of Mefenamic acid was found to be about 3.05 minute. The method is validated as per ICH guidelines. Forced Degradation of Mefenamic Acid suspension and API was carried out by treating Acid, Base, Oxidative and thermal degradation

INTRODUCTION

Mefenamic Acid is a nonsteroidal anti-inflammatory, analgesic and antipyretic drug. [1] It is most commonly used for the management of pain, fever and menstrual pain. Mefenamic acid decreases inflammation and uterine contractions by inhibition of prostaglandin synthesis by blocking of enzyme cyclooxygenase (Cox-1 and Cox-2). [2] IUPAC name of Mefenamic acid is N-(2, 3-dimethylphenyl)-2-aminobenzoic acid.[3] Molecular Formula $C_{15}H_{15}NO_2$, Molecular Weight 241.3. [4]

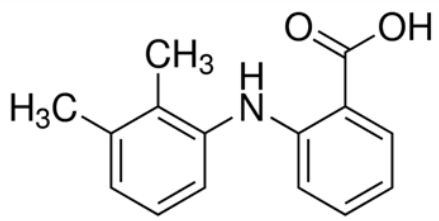

Fig. No. 1: Mefenamic Acid Structure [5]

Stability indicating method must be able to monitor a change in the chemical, physical, and microbiological properties of drug product over time. The ability of the method to monitor a change in the chemical properties of the drug over time, invariably calls for a forced degradation (stress testing) study to be done on the drug substance and drug product. Forced degradation studies may help to facilitate pharmaceutical development as well in areas such as formulation development, manufacturing and packaging, in which knowledge of chemical behavior can be used to improve a drug product. Forced degradation on the drug substance and product will (in addition to establishing specificity) also provide the following information:

1. Determination of degradation pathways of drug substances and drug products;
2. Identify degradation products in formulations that are related to drug substances versus those that are related to non-drug substances (e.g. excipients);
3. Structure elucidation of degradation products;
4. Determination of the intrinsic stability of a drug substance molecule in solution and solid state.
5. Reveal the Acidic, Basic, oxidative, thermal, hydrolytic and photolytic degradation mechanism of the drug substance and drug product.[6]

Stress testing of the drug substance can help identify the likely degradation products, which can in turn help establish the degradation pathways and the intrinsic stability of the molecule and validate the stability indicating power of the analytical procedures used. The nature of the stress testing will depend on the individual drug substance and the type of drug product involved.[7] ICH guideline for stability testing (Q1B) gives guidance in photostability notes that light testing should be an integral part of stress testing. The intrinsic photostability characteristics of new active substances and medicinal products should be evaluated to demonstrate that, light exposure does not result in unacceptable change.[8] ICH Q2 guideline gives guidance to validate analytical mythology in which Specificity is the key factor to determine whether analytical method is stability indicating or not.[9] Forced degradation factors include acidic, base, hydrolysis, thermal degradation, photolysis and oxidation.[10]

MATERIALS AND METHODS

Materials

Potassium dihydrogen orthophosphate, Orthophosphoric acid, Sodium Hydroxide, Methanol, Acetonitrile, Hydrochloric acid, Hydrogen Oxidative, Mili Q water, Mefenamic Acid working standard and API, Meftal-P Suspension (Blue Cross Laboratories Pvt. Ltd.)

Instruments

HPLC (Waters), UV Spectrophotometer (Shimadzu), Analytical balance (Sartorius), Sonicator (PCi Analytics), Magnetic Stirrer (REMI), pH meter (Lab India), Centrifuge (REMI)

Column

Hypersil BDS C18 (250x4.6mm, 5µ particle size).

Method Development

Determination of wavelength

The 20µg/ml solution of Mefenamic Acid was scanned in scale of 200-400nm in UV spectrophotometer and λ max observed at 285nm.

Preparation of Buffer Solution

Dissolve 2.72 gm Potassium dihydrogen orthophosphate in 1000ml water adjust the pH to 3.0 with orthophosphoric acid. Filter through 0.45µ Nylon membrane filter.

Mobile Phase Preparation

Prepare a mixture of Buffer pH 3.0 and acetonitrile in proportion of 20:80 % v/v, respectively. Mix well and Sonicate for 15 minutes.

Diluent

Dissolve 0.4 gm of sodium hydroxide into 1000ml methanol.

Preparation of Standard Solution

Weigh accurately about 20 mg Mefenamic acid working standard into 100 ml volumetric flask, add 70 ml diluent, sonicate to dissolve contents completely. Make up the volume upto the mark with diluent.

Dilute 5 ml of above solution to 50ml with mobile phase and shake well.

Preparation of Sample Solution

Weigh accurately about 5ml of suspension (eq. to 100 mg of Mefenamic Acid) into 100 ml volumetric flask, add about 70 ml of diluent and sonicate for 15 minutes and allow to cool to room temperature, make up the volume upto the mark with diluent and mix well. Filter the solution through Whatman No. 1.

Dilute 2 ml of filtrate to 100ml with mobile phase and shake well.

Preparation of Placebo Solution

Weigh accurately about 5ml of placebo into 100 ml volumetric flask, add about 70 ml of diluent and sonicate for 15 minutes and allow to cool to room temperature, make up the volume upto the mark with diluent and mix well. Filter the solution through Whatman No. 1.

Dilute 2 ml of filtrate to 100ml with mobile phase and shake well.

Chromatographic Conditions

The separation of Mefenamic Acid was carried out on Hypersil BDS C18 (250x4.6mm, 5µ particle size) at flow rate of 1.5 ml/min. Injection volume kept 10µL, detection wavelength is 285nm.

Standard solution and sample solution was prepared by using above procedure and chromatograms were recorded. The retention time of Mefenamic Acid was found to be about 3.05 minutes.

Method Validation

The method was validated as per ICH guidelines for Specificity by forced degradation, Precision, Linearity and Range, Accuracy, Robustness, Filter study, Solution Stability.

Forced Degradation Study (Specificity)

Forced degradation study was carried out on Mefenamic Acid under various conditions explained in ICH guideline QA (R2), such as, acidic, basic, oxidative and thermal degradation. Mefenamic Acid API, Mefenamic Acid Suspension and Placebo subjected to stress conditions as per explained in Table No.1.

Table No. 1

Type of Degradation	Degrading Agent	Temperature	Time of Exposure
Acidic	5N HCl	100°C	2 Hrs.
Basic	5N NaOH	100°C	5 Hrs.
Oxidative	30% H_2O_2	100°C	4 Hrs.
Thermal	Temperature	80°C	6 Hrs.

Control Sample

Weigh accurately about 5ml of suspension (eq. to 100 mg of Mefenamic Acid) into 100 ml volumetric flask, add about 70 ml of diluent and sonicate for 15 minutes and allow to cool to room temperature, make up the volume upto the mark with diluent and mix well. Filter the solution through Whatman No. 1.

Dilute 2 ml of filtrate to 100ml with mobile phase and shake well.

Acidic Degradation

Weigh accurately about 5ml of suspension (eq. to 100 mg of Mefenamic Acid) into 100 ml volumetric flask, add about 10 ml of diluent and 10ml of 5N Hydrochloric Acid, heat on a water bath at temperature 100°C for 2 Hrs. allow to cool and neutralize the above mixture with 5 N sodium hydroxide transfer the neutralized sample to 100ml volumetric flask add diluent upto 70 ml volume and Sonicate for 15 minutes and allow to cool to room temperature, make up the volume upto the mark with diluent and mix well. Filter through Whatman No.1 filter.

Dilute 2 ml of filtrate to 100ml with mobile phase and shake well.

A blank, placebo and API should be treated in same manner.

Basic Degradation

Weigh accurately about 5ml of suspension (eq. to 100 mg of Mefenamic Acid) into 100 ml volumetric flask, add about 10 ml of diluent and 10ml of 5N sodium hydroxide, heat on a water bath at temperature 100°C for 5 Hrs. allow to cool and neutralize the above mixture with 5 N Hydrochloric Acid transfer the neutralized sample to 100ml volumetric flask add diluent upto 70 ml volume and sonicate for 15 minutes and allow to cool to room temperature, make up the volume upto the mark with diluent and mix well. Filter, dilute 2 ml of filtrate to 100ml with mobile phase and shake well.

A blank, placebo and API should be treated in same manner.

Oxidative Degradation

Weigh accurately about 5ml of suspension (eq. to 100 mg of Mefenamic Acid) into 100 ml volumetric flask, add about 50 ml of diluent and 10ml of 30% Hydrogen oxidative solution, heat on a water bath at temperature 100°C for 4 Hrs. allow to cool upto room temperature upto add diluent 70 ml volume and sonicate for 15 minutes and allow to cool to room temperature, make up the volume upto the mark with diluent and mix well. Filter, dilute 2 ml of filtrate to 100ml with mobile phase and shake well.

A blank, placebo and API should be treated in same manner.

Thermal Degradation

Weigh accurately about 5ml of suspension (eq. to 100 mg Mefenamic Acid) into a 100ml volumetric flask, keep in oven at temperature 80°C for 6 Hrs., allow to cool, add 70ml diluent and sonicate for about 15 minutes and allow to cool to room temperature, Make up the volume up to the mark with diluent and allow the solution to settle down about 10 min. pass a portion of above solution through a Whatman No-1 Filter.

Dilute 2ml of filtrate to 100 ml with mobile phase and shake well.

A blank, placebo and API should be treated in same manner.

Precision

The repeatability was checked by injecting six samples set in optimized chromatographic conditions. Intermediate precision was done by different analyst, different days and different HPLC.

Linearity and Range

Linearity of Mefenamic Acid in standard solution from 20% to 160% i.e. 4 ppm to 32 ppm. The value of coefficient correlation should be less than 0.999.

Accuracy

Accuracy of method was determined at 3 different levels i.e. 50% 100% and 150% of increments in placebo. The recovery at each level and % mean recovery calculated. The % recovery at each level should be in between 98% to 102%.

Robustness

Robustness of method was verified by deliberately varying instrumental conditions such as pH of buffer (±0.2) and flow rate (±0.2ml/min). The % RSD of Method precision sample and robustness sample should not more than 2.0.

Solution Stability

A sample solution containing Mefenamic Acid in diluent should be injected in well equilibrated chromatographic system at time intervals of 0.00 Hrs. and 24.00 Hrs.

respectively. The difference of % assay between initial sample and 24 Hrs. sample should not more than 2.0.

Filter Study

The filter study was performed to check suitability of different filters with filter specified in method. Difference of % assay between filter specified in method and other filters/centrifuge sample should not more than 2.0.

Result and Discussion

Proposed study describes stability indicating liquid chromatographic method for estimation of Mefenamic acid in liquid dosage form. The method optimized and validated as per ICH guidelines.

Forced Degradation (Specificity)

The degradation study indicated that the drug degrades as the decreased areas in the peaks when compared with peak areas of the same concentration of the non-degraded drug, without giving any additional degradation peaks. Percent degradation was calculated by comparing the areas of the degraded peaks in each degradation condition with the corresponding area of the peak of Mefenamic Acid under non degradation condition. For forced degradation with 5 N HCl at 2 Hrs., 5 NaOH at 5 Hrs, 30% v/v H_2O_2 for 4 Hrs. at 100° and thermal degradation for 6 Hrs. at 80°C were done. The % degradation was found to be 5% to 20% for Mefenamic Acid in suspension and API in Acid and Oxidative degradation condition using developed HPLC method. Summary of degradation studies for API and Suspension is given in Table No. 2 and Table No. 3.

For API

Table No. 2

Degradation Condition	% Assay	% Degradation
Control API	99.40	-
Acid	82.79	16.61
Basic	98.58	0.82
Oxidative	93.25	6.15
Thermal	99.05	0.35

For Sample

Table No. 3

Degradation Condition	% Assay	% Degradation
Control Spl	101.82	-
Acid	87.90	13.92
Basic	101.64	0.18
Oxidative	96.52	5.30
Thermal	101.52	0.30

Chromatograms

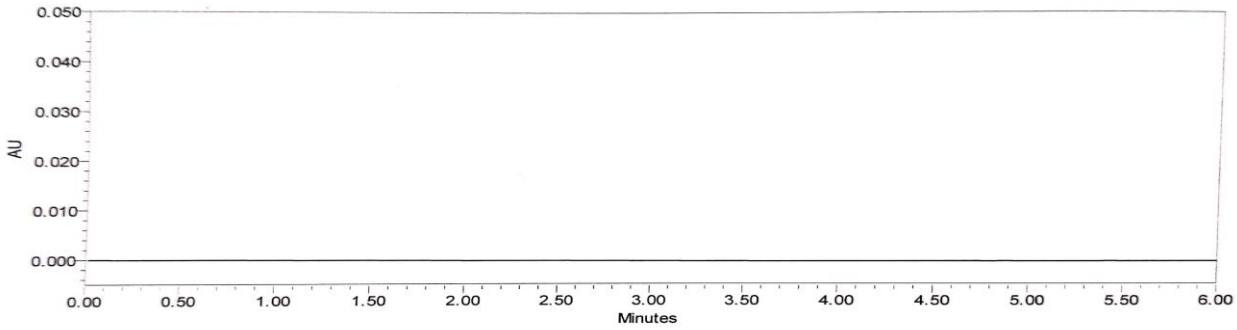

Fig. No. 2: Blank Chromatogram

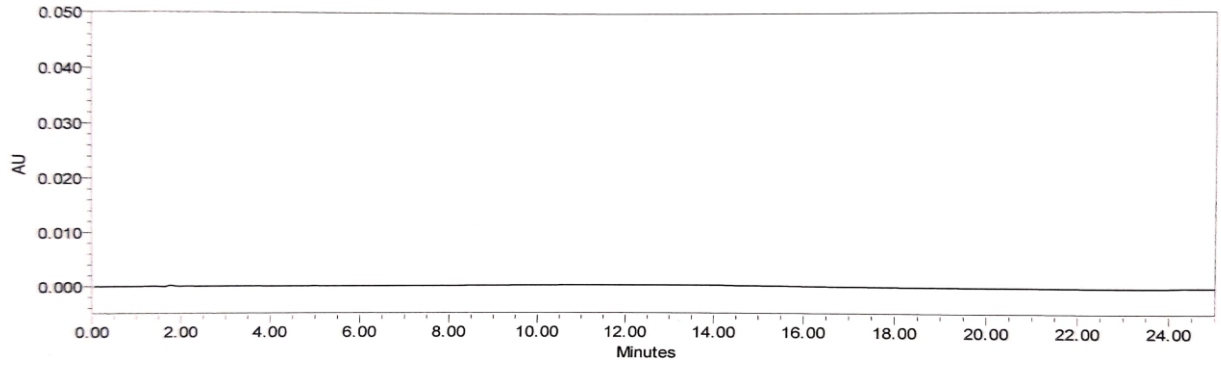

Fig. No. 3: Placebo Chromatogram

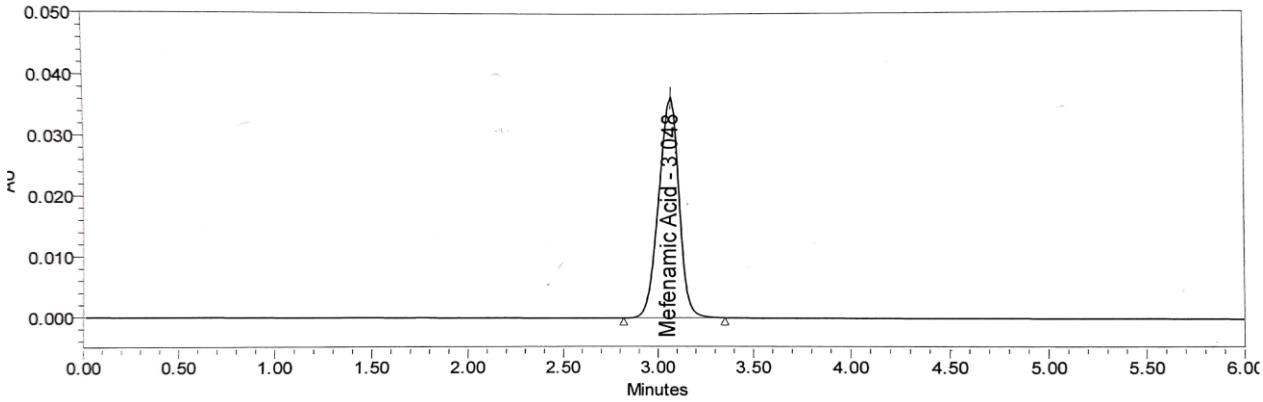

Fig. No. 4: Standard Chromatogram Control

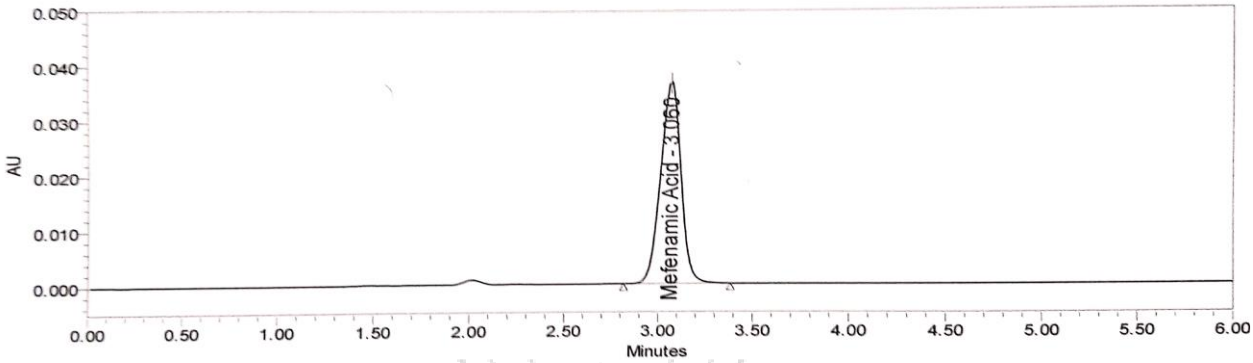

Fig. No. 5: Sample Chromatogram Control

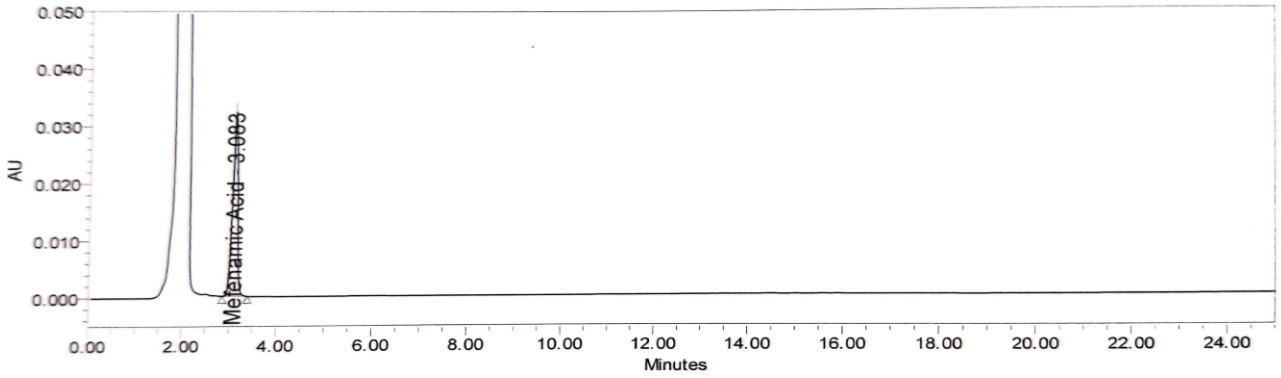

Fig. No. 6: Acidic Degradation Sample Chromatogram

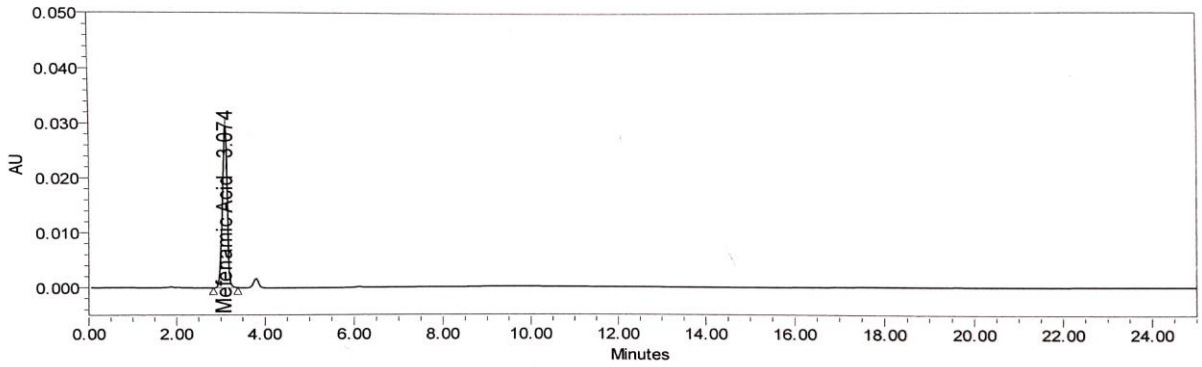

Fig. No. 7: Acidic Degradation API Chromatogram

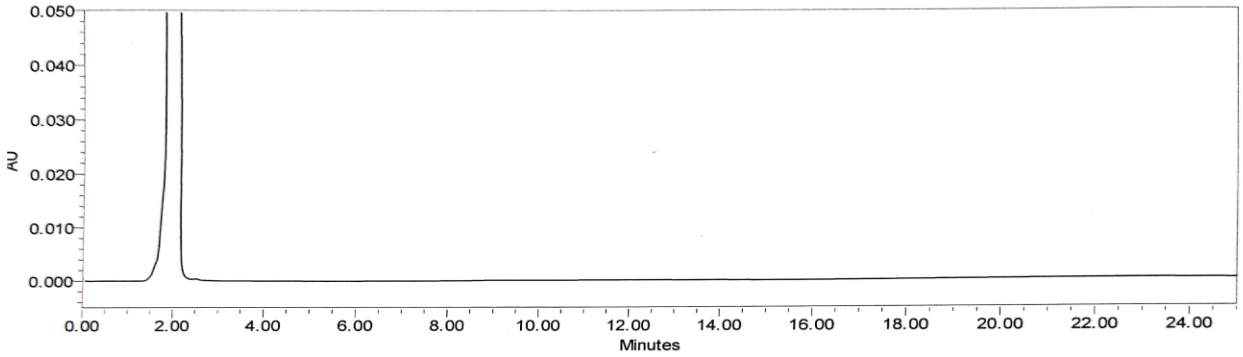

Fig. No. 8: Acidic Degradation Placebo Chromatogram

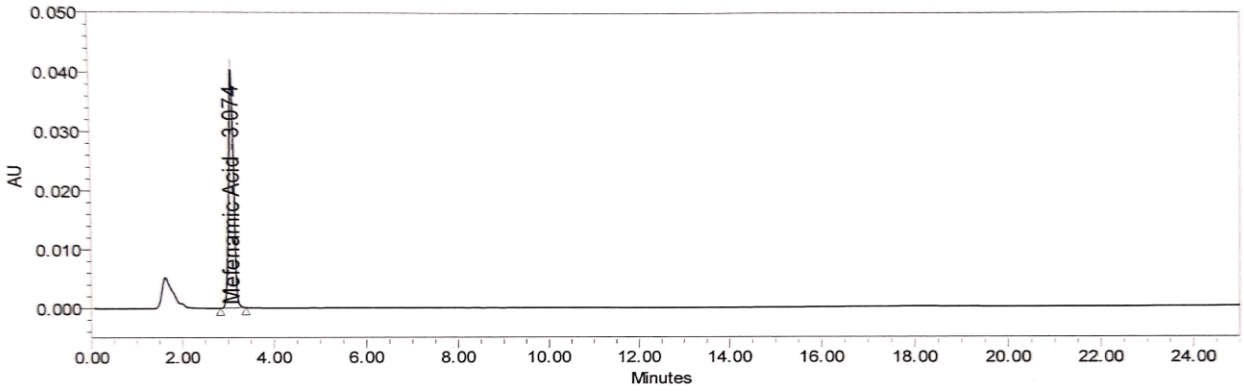

Fig. No. 9: Basic Degradation Sample Chromatogram

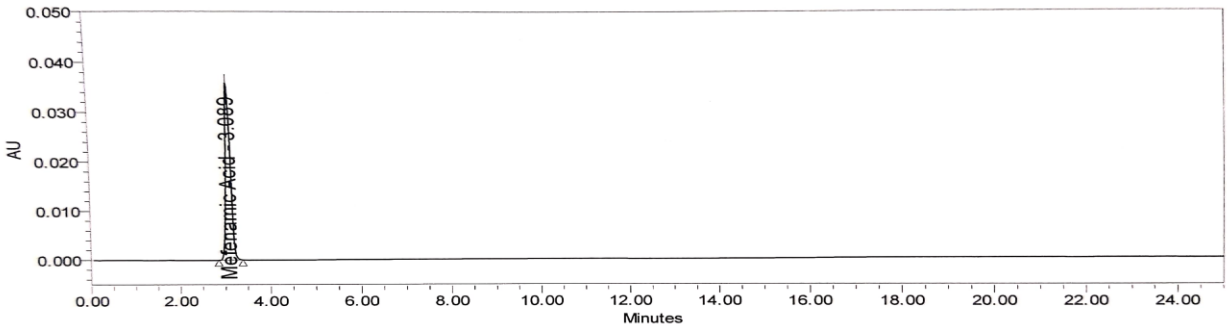

Fig. No. 10: Basic Degradation API Chromatogram

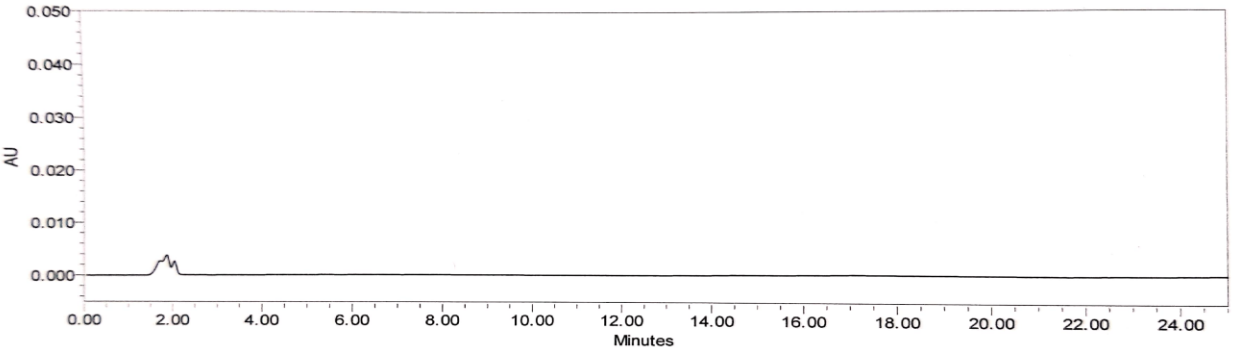

Fig. No. 11: Basic Degradation Placebo Chromatogram

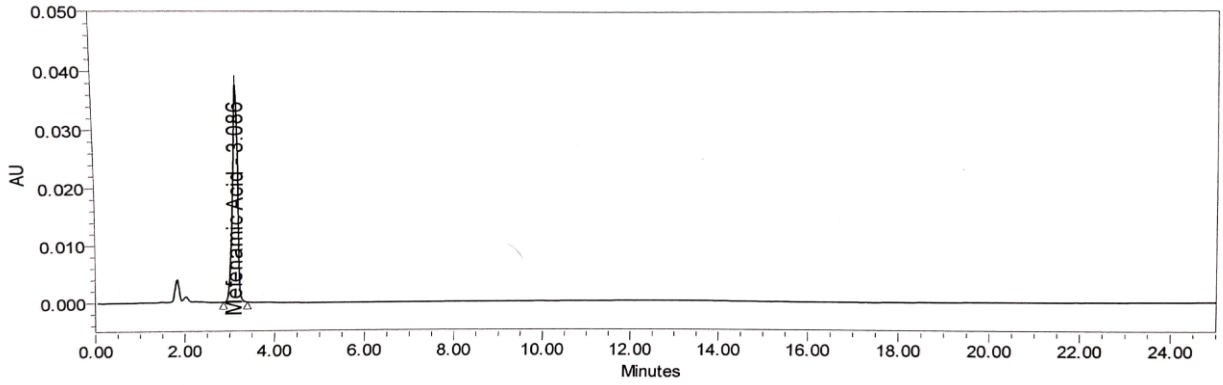

Fig. No. 12: Oxidative Degradation Sample Chromatogram

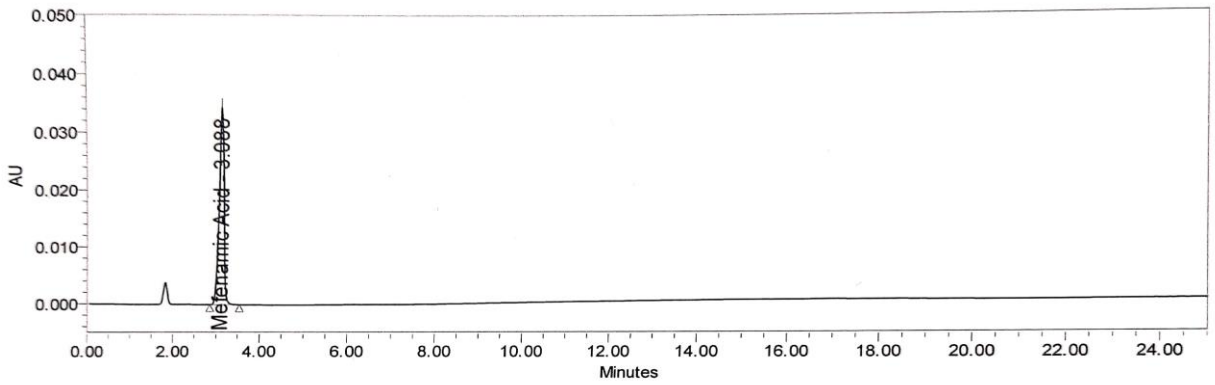

Fig. No. 13: Oxidative Degradation API Chromatogram

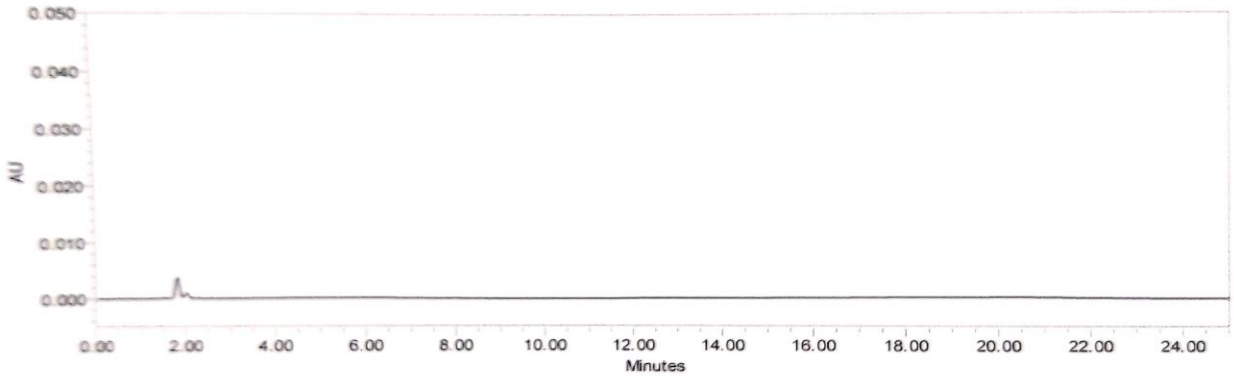

Fig. No. 14: Oxidative Degradation Placebo Chromatogram

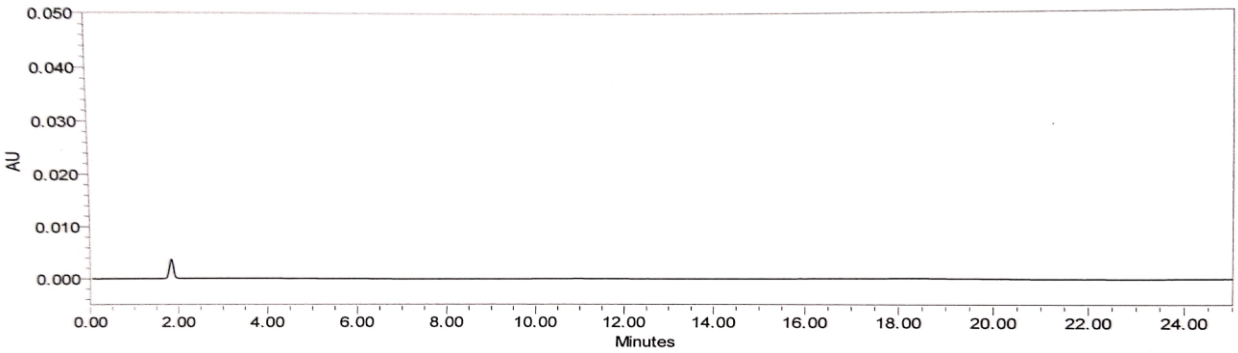

Fig. No. 15: Oxidative Degradation Blank Chromatogram

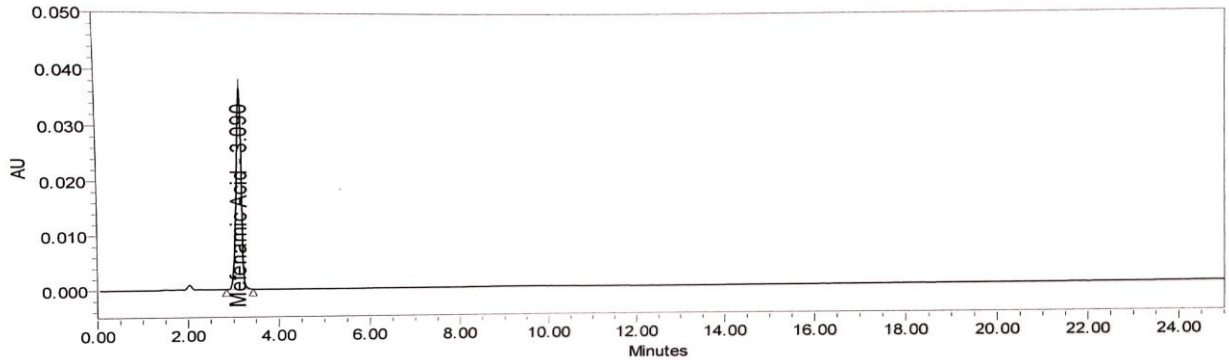

Fig. No. 16: Thermal Degradation Sample Chromatogram

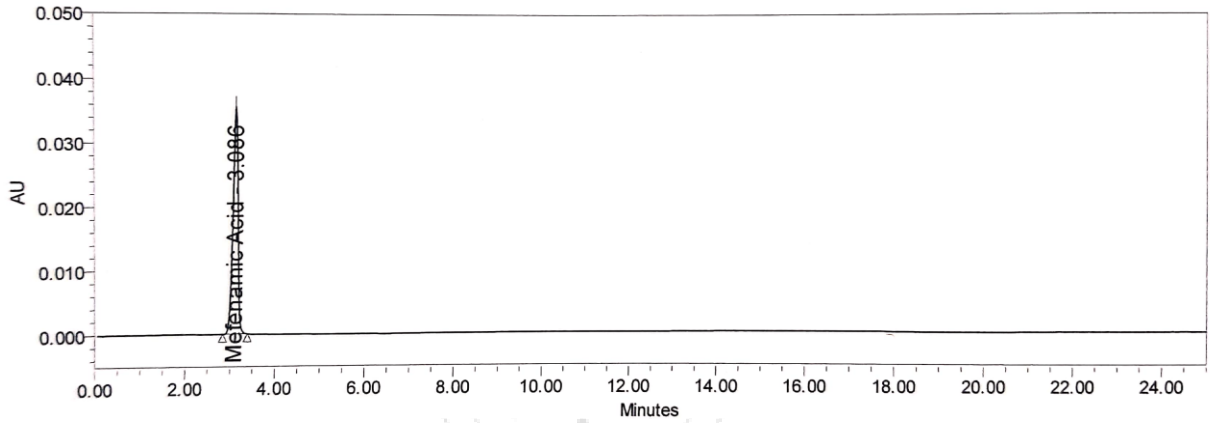

Fig. No. 17: Thermal Degradation API Chromatogram

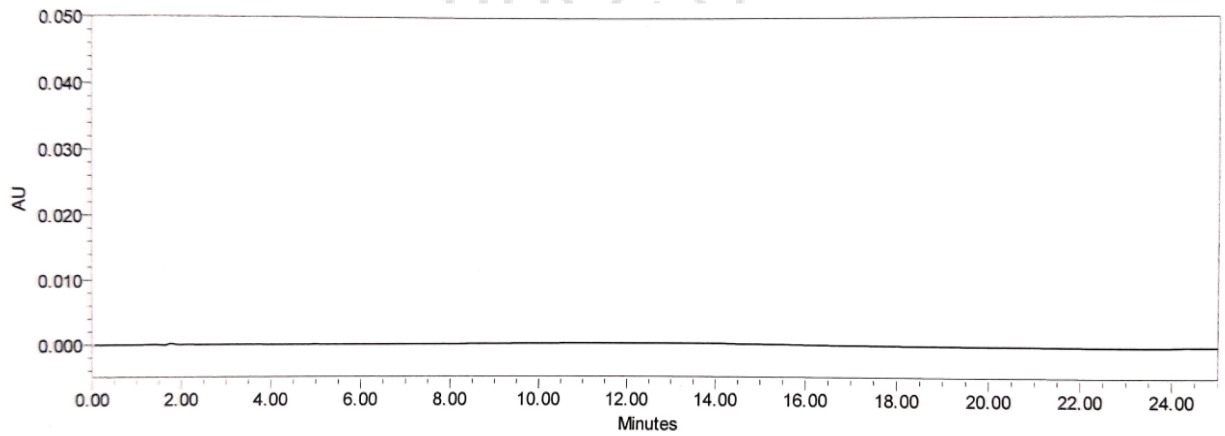

Fig. No. 18: Thermal Degradation Placebo Chromatogram

Precision

System Suitability

A Standard solution of Mefenamic Acid was prepared and injected 5 times into well-equipped chromatographic system. The RSD of peak areas of 5 replicate injections found less than 2.0%, tailing factor was less than 2.0 and theoretical plates found more than 2000.

Table No. 4

Parameter	**Limit**	**Result**
% RSD of Area	NMT 2.0%	0.47
Tailing factor	NMT 2.0	1.21
Theoretical Plates	NLT 2000	7163

Method Precision (Repeatability)

Six Homogeneous samples were prepared and injected into well-equipped chromatographic system. The RSD of % assay of 6 samples found less than 2.0%.

Table No. 5

Sample No	**% Assay**
1	99.13
2	98.96
3	98.58
4	99.01
5	99.07
6	99.16
Avg	**98.99**
SD	**0.21**
% RSD	**0.21**

Intermediate Precision (Ruggedness)

Intermediate precision was done by different analyst in different day on different HPLC instrument. Six samples prepared and injected by different analyst in different well

equilibrated HPLC system. % RSD of assay of 12 samples (6 from method precision and 6 from intermediate precision) found less than 2.0.

Table No. 6

Sample		**% Assay**
Method Precision sample	1	99.13
	2	98.96
	3	98.58
	4	99.01
	5	99.07
	6	99.16
Intermediate Precision sample	1	99.56
	2	99.02
	3	99.26
	4	98.62
	5	98.48
	6	99.16
Average		**99.0**
SD		**0.31**
% RSD		**0.31**

Linearity and Range

The linearity graph was plotted by taking concentration on X-axis and peak area on Y-axis. The calibration curve showed linearity in the range of 4 ppm to 32 ppm for Mefenamic Acid, coefficient correlation found 1.0.

Table No. 7

Linearity Level	**Concentration (µg/ml)**	**Mefenamic Acid Area**
20	4	51751
40	8	103481
60	12	154597
80	16	207565
100	20	261304
120	24	312035
140	28	365612
160	32	418952

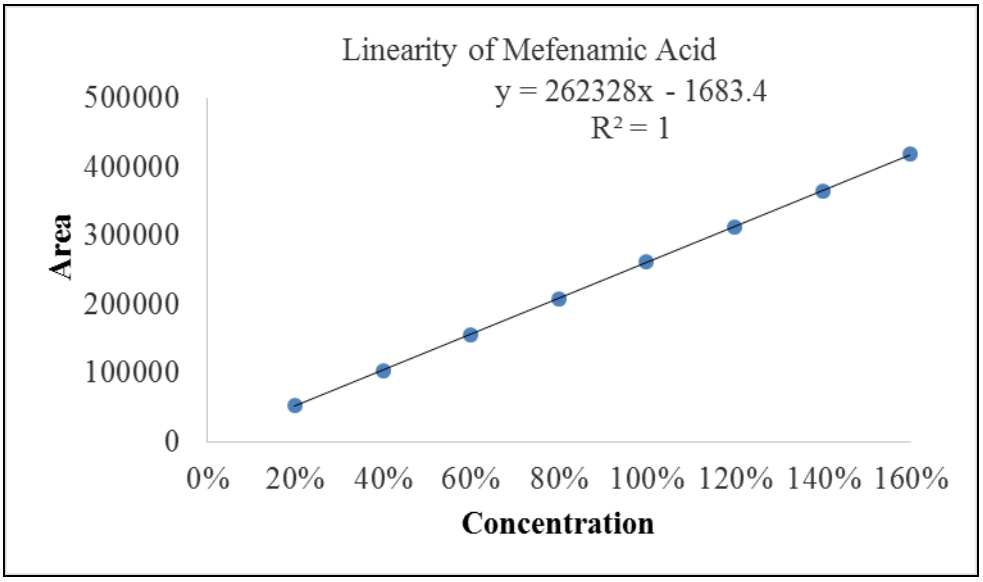

Fig. No. 19

Accuracy

Accuracy for Mefenamic Acid was performed at 3 levels i.e. 50%, 100% and 150%. At each level sample prepared in triplicate and injected. The % recovery found at each level was found in between 98% to 102% with % RSD less than 2.0.

Table No. 8

Mefenamic Acid Recovery				
Level (%)	**Amount added (µg/ml)**	**Amount found (µg/ml)**	**% Recovery**	**Statistical Analysis**
50	10.04	10.07	100.30	Avg=99.67 SD=0.56 % RSD=0.57
	10.08	10.03	99.50	
	10.10	10.02	99.21	
100	20.03	20.20	100.85	Avg=100.07 SD=0.71 % RSD=0.71
	20.09	20.07	99.90	
	20.02	19.91	99.45	
150	30.05	29.83	99.27	Avg=99.88 SD=0.57 %RSD=0.57
	30.08	30.07	99.97	
	30.12	30.24	100.40	

Robustness

In robustness minor changes done in flow rate (1.5±0.2ml/min) and pH of buffer (3.0±0.2). Duplicate samples analysed and calculated for each altered condition. The % RSD of six

method precision samples and 2 robustness samples (n=8) for each condition found less than 2.0.

Table No. 9

Mefenamic Acid		
Parameter	**Assay (n=8)**	**% RSD (n=8)**
pH Plus 3.2	99.74	0.07
pH Minus 2.8	99.00	0.91
Flow Plus 1.7 ml/min	99.52	0.05
Flow Minus 1.3 ml/min	99.13	0.40

Solution Stability

A sample solution injected in a well equilibrated chromatographic system at time intervals, Initial sample and 24 Hrs sample. The difference of % assay between initial sample and 24 Hrs sample is found less than 2.0.

Table No. 10

Mefenamic Acid	
Time (in Hrs)	**% Assay**
Initial Sample	99.41
24 Hrs Sample	99.35
Difference	0.06

Filter Study

In filter study same sample solution through different filters/centrifuge and injected in well-equipped chromatographic system to check compatibility of different filters/ centrifuge. The difference of % assay between Whatman No. 1filter paper, 0.45µ Nylon syringe filter, Whatman No. 41 filter paper and centrifuge found less than 2.0. Hence % assay does not affected by using above filters/centrifuge.

Table No. 11

Mefenamic Acid		
Type of filter	**% Assay**	**Difference**
Whatman No. 1	100.19	--
Whatman No. 41	98.55	1.64
0.45µ Nylon Syringe filter	98.50	1.69
Centrifuge	98.64	1.55

CONCLUSION

A simple, accurate, precise, stability indicating reverse phase chromatographic method has been developed for estimation of Mefenamic Acid in suspension dosage form.

Validation of method proved that the method is specific and suitable for analysis of Mefenamic Acid suspension without any interference from common excipients or potential degradation product of Mefenamic Acid and excipients.

The developed method can be used for routine analysis and stability samples of Mefenamic Acid suspension.

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