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Stability Indicating Method for the Determination of Mefenamic Acid in Pharmaceutical Formulations by HPLC

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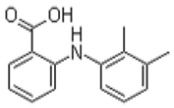
ABSTRACT

A simple, RP-HPLC stability indicating method was developed for determination of mefenamic acid in pharmaceutical formulations and its degradation products using C8 column with the mobile phase containing mixture of Buffer : Acetonitrile + THF in the ratio of 55:45 v/v at a flow rate of 1.0 ml/min was found to yield satisfactory retention time of about 18.253 min with sharp symmetrical peak at a detection wavelength of 285 nm. The method was validated using ICH guidelines and was found to be linear in the range 0.5-2 µg/ mL. The proposed method shows good separation of mefenamic acid and its degradation products. The developed method can be applied successfully for the determination of mefenamic acid and its degraded products.

INTRODUCTION

Mefenamic acid belongs to the category of nonsteroidal anti-inflammatory, analgesic, antipyretic agent with molecular formula $C_{15}H_{15}NO_2$ (M.W. 241,29) and chemical name as 2-(2,3-dimethylphenyl)aminobenzoic acid (Martindale, 2007; MHRA 2007; CSM & MCA; 1993, ICH 1996). The chemical structure of Mefenamic acid is depicted below.



Chemical structure of Mefenamic acid

Mefenamic acid is commonly used for the management of pain, fever and menstrual pain. Mefenamic acid decreases inflammation and uterine contractions by the inhibition of prostaglandin synthesis by competitive blocking of the enzyme cyclooxygenase (COX) (MHRA 2007; CSM & MCA 1993).

The literature survey revealed the analysis for Mefenamic acid using HPLC/UV with chromatographic separation performed on a C_{18} column (250 × 4.6 mm I.D.) and with the employed mobile phase of acetonitrile–water (50:50, v/v, pH 3) or using 10 mM phosphoric acid -acetonitrile (40:60, v/v) at the detection wavelength of 280 nm (Maron & Wright, 1990; Goyal & Singhvi 2008; Dahivelkar et al 2000; Rouini et al 2004a).

However, no study till date has performed the HPLC determination of mefenamic acid with the mobile phase employed in the present investigation. Moreover, recently simultaneous determination of drotaverine HCL and mefenamic acid and its stress degradation products was decribed using some other mobile phase (Krishnan et al 2013).

However, the stability indicating method of the mefenamic acid alone with the adopted mobile phase in this investigation has not yet been performed. Therefore, the present study aims to develop a stability indicating method for mefenamic acid using RP-HPLC technique.

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MATERIAL AND METHODS

Chemicals and reagents

The standards drug of Mefenamic acid with stated purity of 99.88 % was obtained from Torrent Pharmaceuticals, India. The marketed tablet preparation with brand name of Meftal (Blue Cross Laboratories) label claimed 250 mg was used in this study. Acetonitrile, THF (HPLC grade), ammonium dihydrogen orthophosphate, ammonia, sodium hydroxide, hydrochloric acid and hydrogen peroxide (analytical reagent grade) were procured from Merck Pvt. Ltd.

Double distilled water (HPLC grade) was used throughout the analysis. Mobile phase was filtered using 0.45 µm nylon filters by Millipore (USA).

Equipment and chromatographic conditions

The HPLC system of Shimadzu, LC-2010 CHT consisted Pump-LC-20 ATvp, and Detector-SPD-20 A VP with Software-LC solution and a Rheodyne injection valve with a 20 µL loop. Peak areas were integrated using a Shimadzu LC solution software program. The experimental conditions were optimized on Princeton SPHER-100 C_8 column 100A (250 x 4.6 mm, 5 µm) at room temperature.

The chromatographic separation of Mefenamic acid and its degradation products were carried out on reverse phase C_8 column by using various mobile phase but the mobile phase containing mixture of mixture of buffer : acetonitrile + THF in the ratio of 55:45 v/v at a flow rate of 1.0 ml/min was found to yield satisfactory retention time of about 18.253 min with sharp symmetrical peak at a detection wavelength of 285 nm and was found satisfactory for the resolution of degradation products from the drug.

Standard solutions

Solution A (stock standard solution)

An accurately weighed quantity of about 20.0 mg of was Mefenamic acid dissolved in mobile phase and diluted to 100.0m1.

Solution B (working standard solution)

A 1.0 ml of solution A was diluted to 10.0 ml with mobile phase (concentration 200 µg/ml).

Solution C (working standard solution)

A 1.0 ml of solution B was diluted to 10.0 ml with mobile phase (concentration 20 µg/ml).

Preliminary optimization of mobile phase and other chromatographic conditions

Using following chromatographic parameters, different mobile phases were tried to get retention time for parent drug. The chromatographic conditions were set as per the given parameters and mobile phase was allowed to equilibrate with stationary phase as was indicated by the steady baseline. Solution C was injected in the Rheodyne injector (10µl) and the chromatograms were recorded for the drug. Various mobile phases were tried by permutation and combination and also by varying the flow rate and column temperature. However, the mobile phase containing mixture of buffer: acetonitrile + THF in the ratio of 55:45 v/v at a flow rate of 1.0 ml/min was found to yield satisfactory retention time of about 18.253 min with sharp symmetrical peak. The chromatogram is depicted in Fig. 1.

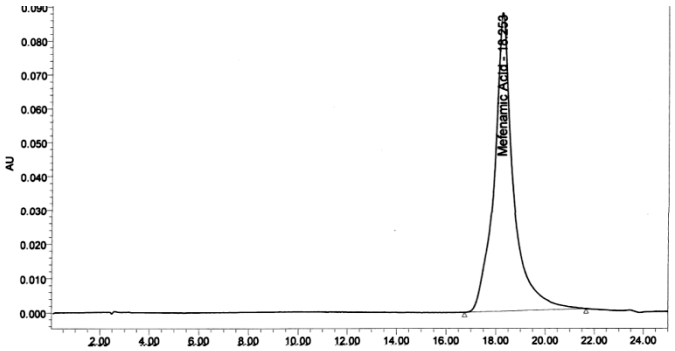

Fig. 1: HPLC chromatogram of Mefenamic acid.

Preparation of mobile phase

Mobile phase was prepared by dissolving 5.75 g of ammonium dihydrogen orthophospahte in 1000 ml water and pH adjusted to 5.0 with ammonia. Obtained solution was filtered by 0.45µ filter, 55 ml of this was added to 45 ml of acetonitrile and THF mixture (460:140).

Force degradation (Stress studies) of Mefenamic acid

The stress studies of Mefenamic acid were performed by various stress conditions to study the effect over wide range of pH, heat, oxidation and photo degradation as per the ICH guidelines

Acid degradation

20 mg of Mefenamic acid was dissolved in 20 m1 of diluent and 10 ml of 1 N hydrochloric acid (with 1 mg/ml concentration of drug) and 25 ml of it was refluxed in round bottom flask on boiling water bath for 8 hr. The remaining solution was kept at room temperature.

Alkali degradation

20 mg of Mefenamic acid was dissolved in 20 m1 of diluent and 10 ml of 0.1N NaoH (1 mg/ml) and 25 ml of it was refluxed in round bottom flask on boiling water bath for 8 hr. The remaining solution was kept at room temperature.

Oxidative degradation

20 mg of Mefenamic acid was dissolved in 20 m1 of diluent and 10 ml of 30 % H_2O_2 (1 mg/ml) and 25 ml of the above solution was refluxed in round bottom flask on boiling water bath for 45 min, 2 or 6 hr. The remaining solution was kept at room temperature.

Photo degradation

20 mg of Mefenamic acid was evenly spread in thin layer in a covered petridish were kept in sunlight for different time intervals and also in dark as a blank.

Thermal degradation

20 mg of Mefenamic acid in different weighing bottles were kept at 105° for different time intervals.

Sample preparation and HPLC resolution

The samples (1 ml each) were withdrawn during stress studies every 1st , 3rd, 5th and 8th hr, to study the extent of degradation. To compare the effect of various stress conditions at elevated temperature and room temprature corresponding blanks were kept at room temprature and the samples were withdrawn simultaneously.

The withdrawn samples were diluted to 10 ml with diluent (the samples of acid and alkali degradation were neutralized prior to dilution).The resultant solutions (1 ml each) were further diluted to 10 ml with mobile phase.

In case of thermal degradation studies the samples were withdrawn every 7th, 14th, 30th and 60th day whereas in case of photo degradation the samples were withdrawn every 7th, 14th and 30th day along with a blank kept in dark. In all, these cases the total quantity of each withdrawn sample was dissolved in mobile and diluted to 100 ml. The resultant solutions (1 ml each) were further diluted to 10 ml with mobile phase (to obtain concetrations 10 µg/ml with respect to parent drug stock solution).

The degraded samples so prepared were injected in HPLC and chromatograms were obtained using preliminarily optimized mobile phase. The chromatograms of degradation products are depicted in Fig. 2 to 6.

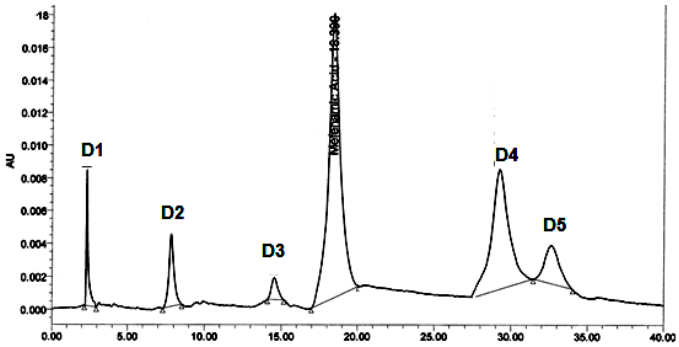

Fig. 2: HPLC Chromatogram of Acidic degradation.

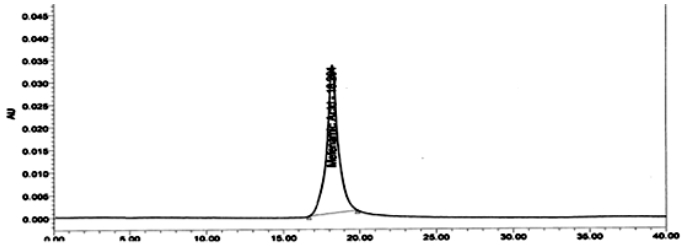

Fig. 3: HPLC Chromatogram of Alkaline Degradation.

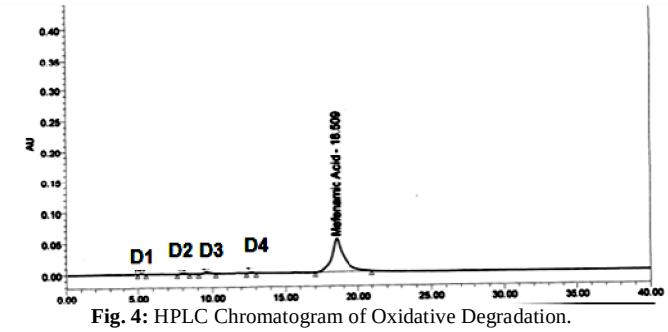

Fig. 4: HPLC Chromatogram of Oxidative Degradation.

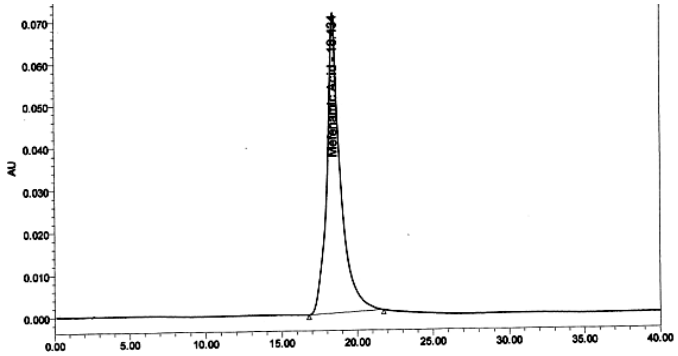

Fig. 5: HPLC Chromatogram of Thermal Degradation.

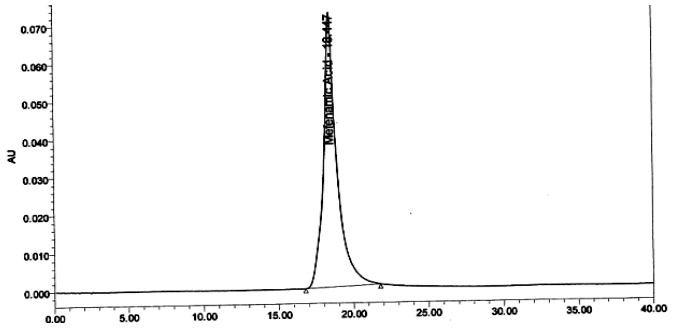

Fig. 6: HPLC Chromatogram of Photo Degradation.

Table 1: Summary of forced degradation study.

Stress conditions	Retention Time (min) (Degradation products)
Acid	D1: 2.313, D2: 7.825, D3:14.531, D4: 29.222, D5: 32.591
Base	No degradation products
Oxidative	D1: 5.206, D2: 8.002, D3:9.571, D4: 12.677
Thermal	No degradation products
Photo	No degradation products

Study of system suitability parameters

The chromatographic conditions were set as per the optimized parameters and mobile phase was allowed to equilibrate with stationary phase as was indicated by the steady baseline. Five replicate injections were made separately and the chromatograms were recorded. One of the standard chromatogram is depicted in Fig.1 and the results of system suitability parameters are given in Table 2 & 3.

Thus, the preliminarily optimized mobile phase is capable of resolving the parent drug mefenamic acid and all the degradation products with adequate system suitability parameters.

Table 2: Repeatability of response.

Sr. No	Replicate injections	Peak Area
1	1	4580792
2	2	4588803
3	3	4599478
4	4	4599833
5	5	4559499
	Mean	4585681
	±SD	16656.7
	% RSD	0.363233

Table 3: System suitability parameters.

Sr. No	Parameters	Results
1	Retention time	18.253
2	Capacity factor	1.20
3	Asymmetry	1.36
4	Theoretical Plate (per meter)	3755

Linearity of response

Aliquot portions of stock solution B were diluted to 10.0 ml with mobile phase. The chromatographic conditions were set as per the optimized parameters and mobile phase was allowed to equilibrate with stationary phase as indicated by the steady baseline. Standard solutions of different concentration were injected separately and the chromatograms were recorded (the curve was found to be liner over a concentration range of 0.5 to 2 µg/ml). A graph was plotted as Peak area vs concentration of drug and is depicted in Fig. 7.

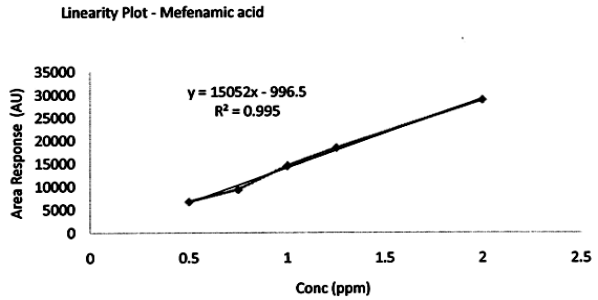

Fig. 7: Linearity of response.

Table 4: Observation and result of estimation of tablets.

Sr. No.	Weight of sample (mg)	Amount of drug estimated (mg/tab)	% Drug estimated
1	17.16	246.88	98.75
2	17.21	244.96	97.98
3	17.31	244.80	97.92
4	17.20	245.71	98.28
5	17.40	245.37	98.14
6.	17.22	245.03	98.01
		Mean	**98.18**
		±S.D.	**0.30**
		% RSD	**0.31**

Estimation of Mefenamic acid in tablets by proposed HPLC method

Twenty tablets were weighed (8.586 g) and average weight (0.429 g) was calculated. An accurately weighed quantity of content equivalent to 10 mg of mefenamic acid was transferred to 100 ml volumetric flask. 50 ml of mobile phase was then added and sonicated for 5 mins to dissolve and made up to mark with the same to yield a final concentration of 10 µg/ml. Five replicate sample solutions were prepared in similar manner. After equilibration of stationary phase, three replicate injection of standard solution and each of five sample solutions were made separately and chromatograms were recorded and amount of drug present in average weight of tablet as per of labeled claim was calculated and is given in Table 4. The chromatogram of tablet is depected in Fig. 8.

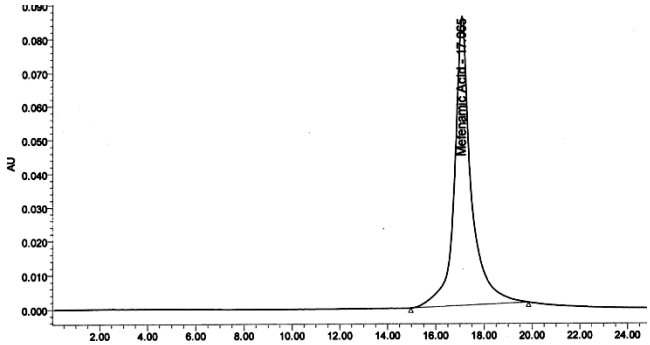

Fig. 8: HPLC chromatogram of tablet.

Validation of proposed HPLC method

Accuracy

Accuracy of the method was determined by investigating the recovery of drug at three levels 50, 100 and 150 µg/mL from placebo mixtures spiked with the API solution. Each concentration was analyzed in triplicate. The mean % recovery was found to be 100.47 %. The results of recovery studies are tabulated in table 5.

Table. 5: Result of recovery studies.

Sr. No	Spiked level (%)	Sample area	µg/ml added	µg/ml found	% Recovery
1	50	6179456	100	299.81	99.91
2	100	8274905	100	201.48	101.07
3	150	10295800	100	249.07	99.77
				Mean	100.47
				±S.D	0.7118
				% RSD	0.7118

Linearity and Range

From the data obtained under accuracy studies, a graph was plotted as percent labelled claim verses peak area which was observed to be linear over 25-125% of labelled claim.

Precision

Precision of analytical method is expressed as SD and RSD of series of replicate measurements. Precision of estimation of mefenamic acid by proposed method was ascertained by replicate analysis of homogeneous samples of tablet powder. Results are as shown in Table 4

Specificity

Accurately weighed quantities of tablet powdered equivalent to about of 20 mg Mefenamic acid were transferred to five different 25.0 ml volumetric flasks. The samples were then exposed to stress conditions for 24 h as follows

- Normal (control)
- At room temp. after addition of 1.0 ml of 0.1N HCl (Acid) for 16 h.
- At 60° after addition of 1.0 ml of 0.1N NaOH (Alkali) for 24 h.
- At room temp. after addition of 1.0 ml of 3% H_2O_2(Oxide) for 16 h.
- At 60° (Dry heat) for 24 h
- Sunlight (UV) for 24 h

After 24 h the flasks were cooled to room temperature, sonicated with 20.0 ml methanol for 15 min and volumes were made up to 25.0 ml. The solutions were then analyzed in similar manner as described under estimation of mefenamic acid in tablets. The results of specificity study are show in Table 6 and no degradation products were observed except under alkali, acidic and oxidative stress condition .

Table 6: Results of specificity studies.

Sr. No.	Sample	% Estimated
1.	Normal	99.99
2.	Acid	77.51
3.	Alkali	93.52
4.	Oxide	92.21
5.	Heat	98.86
6.	Sunlight	100.00

Ruggedness

The studies were carried out for two different parameters i.e. days (intra-day and inter-day) and analysts.

a)Intra-day and inter-day

The samples were analyzed at different times on same day and three different days. The percent of labeled claim was calculated and the results are shown in able 7.

b) Different analyst

The samples were analyzed by two different analysts by the proposed method. The results are shown in Table 7.

Table 7: Results of ruggedness study (intra-day and inter-day).

Sr. No	Observations	% Drug Estimation		
		Intra- day	Inter- day	Different Analyst
1.	I	99.94	100.15	98.86
2.	II	99.91	99.50	99.91
3	III	99.97	99.20	100.81
	Mean	99.94	99.61	99.86
	±S.D.	0.03	0.485	0.672
	% RSD	0.030	0.486	0.673

Robustness

The tablet sample of Mefenamic acid was analyzed using proposed method after a deliberate change in detection wavelength for estimation by $\pm$ 2 nm. Results are as shown in Table 8.

Table 8: Results of robustness (recommended wavelength 285 nm).

Sr. No	Wavelength (±2 nm)	% Estimation
1	283 nm	98.98
2	287 nm	98.54
	Mean	98.76
	±S.D.	0.31
	% RSD	0.31

CONCLUSION

A simple, sensitive, and accurate stability indicating method using reverse phase HPLC was developed for mefenamic acid in pharmaceutical formulations. The proposed method was validated by testing its linearity, accuracy, precision, limits of detection, and quantitation, and specificity. The method proved able to separate the peaks of active pharmaceutical ingredients (APIs) from the degradation products (produced during forced degradation studies). It is also clear from the chromatograms that both the active ingredient peaks under all the stress conditions were free from any sort of degradation impurities. Taken together, these results allow us to conclude that the method can be successfully used for all stability and validation studies. Method was validated as per ICH Q2 (R2) so it can be used by QC department (ICH 1996).

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