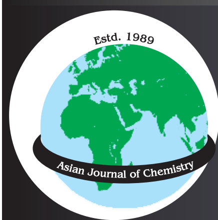

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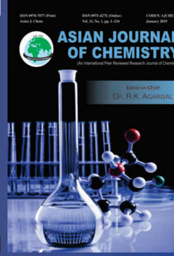

Development and Validation of Stability Indicating RP-HPLC Assay Method for Mefenamic Acid

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The present research work was carried out to evaluate the stability behaviour of mefenamic acid under ICH Q1A (R2) recommended stress conditions. The drug was subjected to hydrolytic, oxidative, photolytic and thermal stress conditions. The drug was found susceptible to degradation under oxidative stress condition but was stable under hydrolytic, photolytic and thermal stress conditions. A total two degradation products were formed, which were separated using HPLC. The chromatographic separation was carried out on Sunfire ODS C-18 (250 × 4.6 mm, 5 μm) column. Optimum resolution was obtained using ammonium dihydrogen phosphate buffer (10 mM, pH 4) and acetonitrile programmed in isocratic elution mode in the ratio of 45:55 v/v at 225 nm using photodiode array detector at a flow rate of 1 mL/min. The designed method was validated as per ICH Q2 (R1) guidelines. The response of drug was linear in the concentration range of 10-100 μg/mL (R^2 = 0.9998). The method was found specific, precise and accurate. The mean accuracy was found to be 100.46 %. The developed method was successfully applied for the analysis of marketed formulation.

Keywords: HPLC, Mefenamic acid, Stability indicating, Stress conditions.

INTRODUCTION

A matter of great concern for pharmaceutical molecule is its chemical stability, which in turn determines the welfare and potency of the drug product. The importance of stability testing profiles of active pharmaceutical ingredients and drug products is held in high esteem by various regulatory bodies like FDA, ICH guidelines, so as to understand the behavior of drug under various stress conditions with respect to time. For filling in registration dossier for new drug moiety, performing stability studies has become mandatory [1,2]. It is very important that the developed stability indicating method should be able to estimate the percentage of unchanged drug during the process when applied for dosage forms. After subjecting the drug to various stress conditions, the samples of drug are utilized for developing the stability indicating method which can be further applied for the testing of drug samples [3,4]. Mefenamic acid, 2-(2,3-dimethyl phenyl)aminobenzoic acid (Fig. 1) is a powerful anti-inflammatory drug used as a effective analgesic and anti-inflammatory agent for various clinical conditions like nonarticular rheumatism, osteoarthritis, sport injuries and other sever musculoskeletal illnesses [5,6]. Various literatures on stability indicating methods for mefenamic acid individually [7,8] and in combination [9-12] with other drugs have been reported. However, few literatures revealed differences in stability studies data of mefenamic acid, indicating different behavior of mefenamic acid in different stress conditions [7,8]. Hence, the purpose of the work was to bring forth an alternative validated RP-HPLC stability indicating method for the estimation of mefenamic acid in pharmaceutical dosage forms.

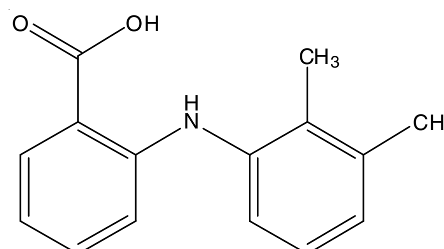

Fig. 1. Chemical structure of mefenamic acid

EXPERIMENTAL

Mefenamic acid was obtained as a gift sample from Blue Cross Ltd. (Goa, India). Acetonitrile (HPLC grade), ammonium

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dihydrogen phosphate, sodium hydroxide, hydrochloric acid and hydrogen peroxide were obtained from Merck (India). HPLC grade water was obtained from Bio-age water purification system. Mefenamic acid with brand name MEFTAL® -250 DT with a label claim of 250 mg drug was purchased commercially. All chemicals were of an analytical grade and used as received. The membrane filters (0.45 μ) were procured from Mecrk, India.

HPLC Instrument and analytical conditions: In current study, Jasco LC-4000 series HPLC system consisting of a quaternary solvent delivery system (PU-4180), an on-line degasser, an auto-sampler (AS-4050), a column temperature controller (CO-4061) and a diode array detector (MD-4010) were used. System control and data analysis were processed with Jasco Chrom NAV software. Other instruments used for this study include Bath sonicator (Citizon Ultrasonic cleaner), Digital Balance (Wensar Digital Electronic Balance) and pH meter (Labtronics, LT-10). The separation and quantification by RP HPLC were achieved on Sunfire ODS C18 column (4.6 × 250 mm, 5 μm) from Waters. The mobile phase used for study involved acetonitrile and 10mM ammonium dihydrogen phosphate buffer (pH adjusted to 4 with dilute acetic acid solution) in the ratio of 55:45 v/v, with flow rate of 1 mL/min. Studies were performed at 40 ºC temperature, with the injected volume of 10 μL and detection wavelength of 225 nm.

Method development

Selection of mobile phase: The objective behind development of simple RP-HPLC method is to study degradation behaviour of mefenamic acid under ICH recommended stress conditions. Different trials were carried out using Sunfire ODS C18 (4.6 × 250 mm, 5 5 μm) column as stationary phase and acetonitrile and ammonium dihydrogen phosphate buffer (pH ranging from 2.5 to 6.5) as a mobile phase.

Preparation of standard stock solution: Stock solution of mefenamic acid was prepared by dissolving accurately weighed 100 mg of mefenamic acid in acetonitrile in 100 mL volumetric flask and making up the mark with acetonitrile. Solutions were filtered through a 0.45 μm membrane filter prior to injection in the system. Twenty tablets of MEFTAL®-250 DT were purchased from the local market, weighed and crushed to a fine powder. Powder equivalent to 50 mg of mefenamic acid was accurately weighed into a 25 mL volumetric flask, made up to volume with acetonitrile, sonicated for 30 min and filtered. The filtrate was diluted to the required concentration with mobile phase before injecting. The solutions were filtered through a 0.45 μm membrane filter before injections.

Calibration curve: Required aliquots were taken from stock solution in 10 mL volumetric flasks and diluted to the mark with mobile phase to get final concentrations of the drug in the range of 10 to 100 μg/mL. 10 μL of each concentration in triplicate were injected and the obtained chromatograms peak areas were recorded. Calibration curves were constructed by plotting the peak area on the y-axis and concentration of the drug on x-axis. The calibration curve was evaluated by its coefficient of determination (R^2).

Method validation: The developed method was validated for selectivity, linearity, accuracy, precision and robustness as per reported method [13].

Linearity: Accurately measured volumes equivalent to 10-100 μg/mL of mefenamic acid were separately transferred from its stock standard solution (1,000 μg/mL) into 10 mL volumetric flasks and the volumes was made up to the mark with mobile phase. Triplet 10 μL injections were given of each concentration. The peak areas were recorded and the calibration curve was plotted.

Accuracy: Accuracy of the proposed method was determined by recovery studies using standard addition method. The percentage recovery studies of mefenamic acid was carried out in triplicate at three different levels 80, 100 and 120 %.

Precision: Intraday repeatability of the method was evaluated by analyzing three concentration of mefenamic acid (30, 50 and 70 μg/mL). Interday precision was evaluated by assaying the chosen concentration of mefenamic acid in triplicates on two successive days using the same procedure stated under chromatographic conditions. The % RSD values were then calculated.

Robustness: The robustness was tested by checking the effect of small deliberate changes in the chromatographic conditions. Changes in the flow rate of mobile phase (± 0.1 unit) and the proportion of organic phase in mobile phase (± 2 % units) on the developed method were studied.

Forced degradation studies: To evaluate the stability indicating properties and specificity of the method, forced degradation studies were performed [14,15]. Drug solution used in stress studies were prepared from stock solution after dilutions and then diluted with mobile phase to give a final concentration of 10 μg/mL. The acidic and alkaline degradations of mefenamic acid were carried out in HCl (1 M) and in NaOH (1M) at 70 ºC over a period of 6 h, respectively. The stressed samples were cooled to room temperature, neutralized and diluted with mobile phase. Neutral hydrolytic degradation was done by subjecting the drug in water for a period of 6 h at 70 ºC. Oxidation of the drug was done by using 10 % H_2O_2 solution for period of 6 days. Photolysis was carried by exposing the drug to direct sunlight for 7 days, whereas thermal degradation was done by heating the drug in oven at 80 ºC for 4 days. The stressed samples obtained in neutral, oxidation, photo and thermal stress conditions were cooled to room temperature and diluted with mobile phase.

RESULTS AND DISCUSSION

Chromatographic separation: Based on various trials conducted drug showed good symmetrical peak with system suitability parameters in acceptable limits when the pH of buffer was adjusted to 4 and the composition of mobile phase was in the ratio of 55:45 % v/v of acetonitrile and buffer, respectively as shown in Table-1. The final optimized chromatographic condition used in the proposed method is listed in Table-2 and the chromatogram obtained with the optimized chromatographic conditions of solution containing 10 μg/mL of mefenamic acid is shown in Fig. 2.

TABLE-1
SYSTEM SUITABILITY PARAMETERS OF THE PROPOSED RP HPLC METHOD

Components	Rt	Area	Peak asymmetry	Theoretical plate
Mefenamic acid (50 μg/mL)	21.10	585330	1.28	17560

TABLE-2
OPTIMIZED CHROMATOGRAPHIC CONDITIONS OF THE PROPOSED RP HPLC METHOD

Mobile phase	Mixture of acetonitrile and 10 mM ammonium dihydrogen phosphate buffer (pH adjusted to 4 with dilute acetic acid solution) in the ratio of 55:45v/v.
Column	Sunfire ODS C18 (250 × 4.6 mm, 5 µm) column
Injection Volume	10 µL
Flow Rate	1 mL/min
Column oven temperature	40 °C
Detection wavelength	225 nm

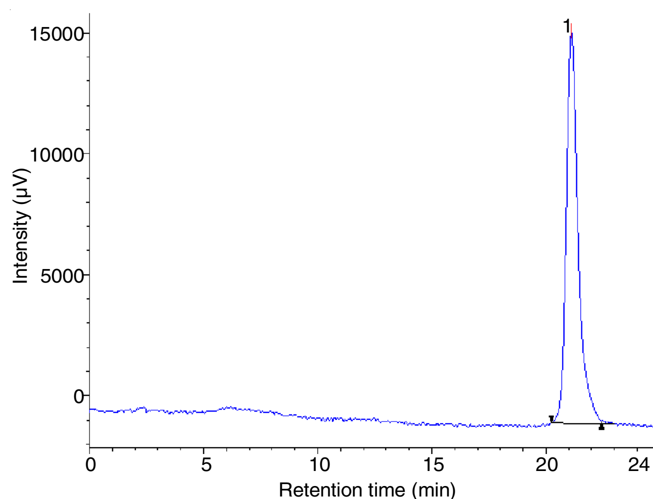

Fig. 2. Optimized chromatogram of mefenamic acid in mobile phase

Calibration curve of mefenamic acid: The correlation coefficient of determination (R^2), slope and intercept for mefenamic acid were 0.999, 52423 and 45446, respectively over the range of 10-100 µg/mL. The calibration curve of mefenamic acid is shown in Fig. 3.

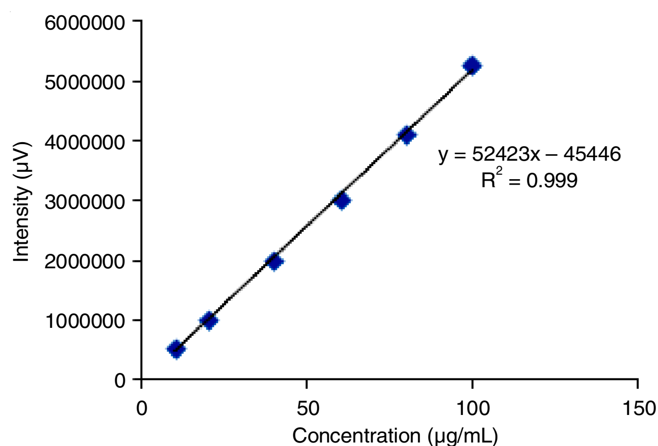

Fig. 3. Standard calibration curve of mefenamic acid

Method validation

Linearity, accuracy and precision: The results indicated that the method is linear over the studied concentration range of 10-100 µg/mL as per calibration curve. Intra and inter-day precision data of the RP-HPLC method for mefenamic acid is shown in Table-3. Results showed good values of % RSD which were within the limits.

The accuracy of the method is determined by recovery studies using standard addition method. The results of accuracy studies are shown in Table-4. The results are within the acceptance criteria of 95-105 % indicating accuracy of method.

TABLE-4
ACCURACY-RECOVERY STUDY OF MEFENAMIC ACID BY STANDARD ADDITION METHOD

Sample No.	Spiked concentration (µg/mL)	Measured concentration (mg/mL)	Recovery (%)
1	32(80%)	32.38	101.18
2	40(100%)	39.98	99.95
3	48(120%)	48.13	100.27

Robustness: Robustness of the method was studied by deliberate variations of the analytical parameters such as flow rate (1 ± 0.1 mL/min) and change in organic phase composition of mobile phase (± 1 %). The results are given in Tables-5.

TABLE-5
RESULTS OF ROBUSTNESS STUDIES OF THE PROPOSED METHOD

Parameter	Variation	Observed value		
		%RSD of area	Tailing factor	Theoretical plates
Flow rate	0.9 mL/min	0.77	1.021	15394
	1.1 mL/min	1.25	1.072	14511
% of organic phase in mobile phase	54 % acetonitrile	0.36	1.042	11590
	56 % acetonitrile	1.13	1.112	9850

Analysis of marketed product: The validated method was applied for the analysis of mefenamic acid tablet. The assay obtained was more than 99 %. Results are summarized in Table-6.

TABLE-6
ASSAY OF MEFENAMIC ACID COMMERCIAL PRODUCT (TABLET)

Sample no.	Formulation	Conc. of sample solution (µg/mL)	Amount found (mg)	Recovery (%)
1	MEFTAL®-250 DT	40	39.60	99.02

TABLE-3
INTRA AND INTER-DAY PRECISION STUDIES OF MEFENAMIC ACID

Sample No.	Conc. (µg/mL)	Intra-day precision		Inter-day precision	
		Mean[a] ± SD	[a]RSD (%)	Mean[a] ± SD	[a]RSD (%)
1	30	1289450 ± 4572	0.35	1287460 ± 3236	0.25
2	50	2038633 ± 8437	0.41	2091966 ± 4785	0.22
3	70	2921366 ± 1236	0.42	2904700 ± 9793	0.33

[a]Mean of 3 replicates

Forced degradation studies

Degradation in acidic medium: During acid hydrolysis, drug was allowed to react with 1M HCl for 6 h. The acid hydrolysis was carried out at room temperature initially, followed by heating with 1 M HCl at 70 ºC for 6 h using constant water bath. Negligible degradation of drug was observed. Chromatogram obtained by degradation of drug in acidic medium is shown in Fig. 4 and the percentage of degradation of drug is shown in Table-7.

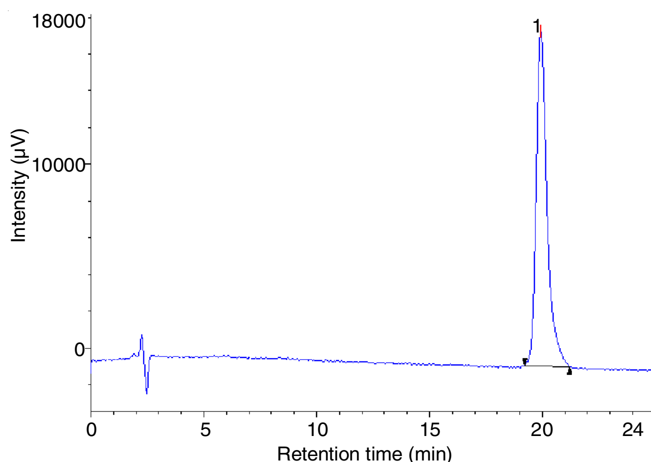

Fig. 4. Representative chromatogram of mefenamic acid on acidic degradation

TABLE-7
RESULTS OF FORCED DEGRADATION STUDIES OF MEFENAMIC ACID

Stress conditions	Drug recovered* (%)	Drug decomposed* (%)
Standard drug	100.0	–
Acidic condition	90.04	9.96
Alkaline condition	92.64	7.36
Neutral condition	93.08	6.92
Oxidative condition	62.56	37.44
Photolytic condition	96.38	3.62
Thermal condition	89.61	10.39

*Mean of three replicates.

Degradation in alkaline medium: The drug was treated with 1 M NaOH initially at room temperature for 6 h, followed by further heating with 1 M NaOH at 70 ºC for 6 h using constant waterbath. Negligible degradation of drug as shown in Table-7 was seen in the chromatogram (Fig. 5) representing degradation in basic medium.

Degradation in neutral medium: Neutral degradation of mefenamic acid was performed using distilled water. The drug was treated with water at room temperature for a period of 6 h followed by heating with water at 70 ºC for 6 h using constant water bath. Table-7 gives the percentage of degradation of drug in neutral medium which showed that the drug is stable in neutral medium and the chromatogram obtained is shown in Fig. 6.

Oxidative degradation: For oxidation, the reagent chosen was hydrogen peroxide (10 %). The drug was made to react with 10 % H_2O_2, for 5 days. In 10 % H_2O_2, decrease in the peak area of the drug was seen from 2nd day onwards. Two degradants peaks were seen in the chromatogram of drug on 3rd day (Fig. 7).

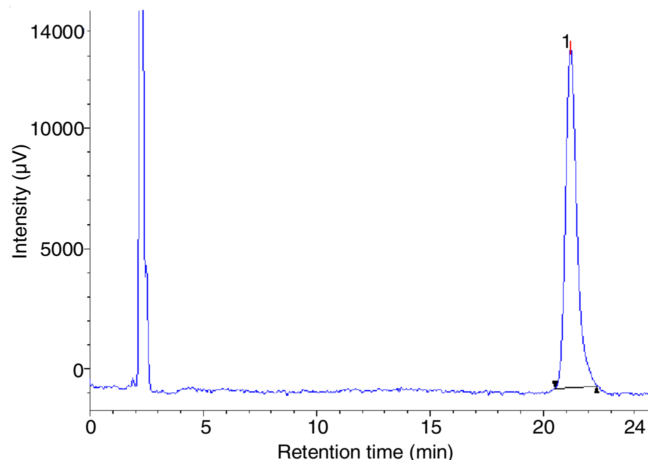

Fig. 5. Representative chromatogram of mefenamic acid on alkaline degradation

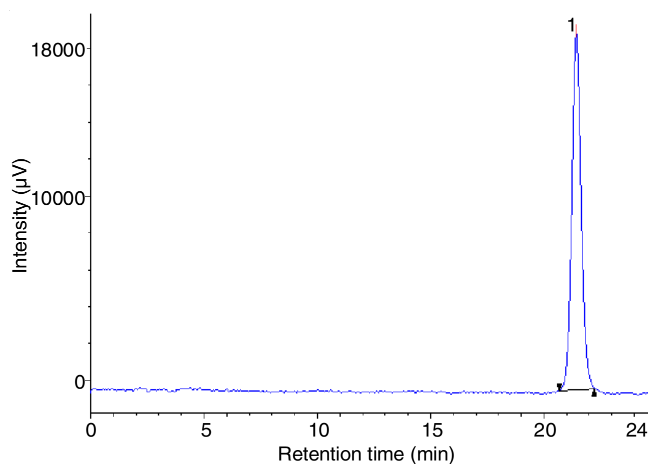

Fig. 6. Representative chromatogram of mefenamic acid on neutral degradation

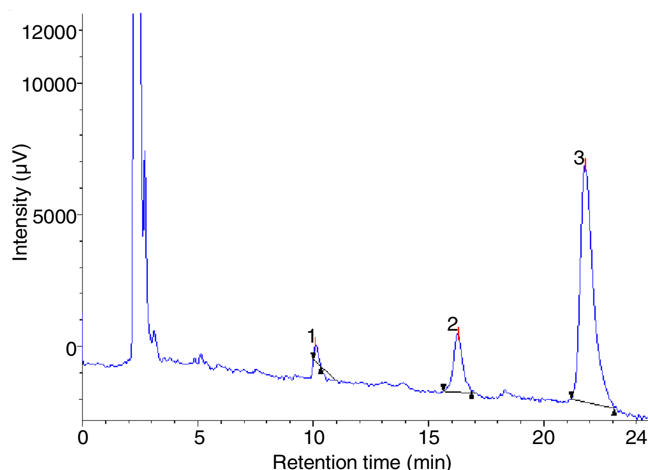

Fig. 7. Representative chromatogram of mefenamic acid on oxidative degradation

Photolytic degradation: The solid drug was exposed to direct sunlight for 7 days. The standard drug was placed in volumetric flask and exposed to sunlight. The chromatogram of sample subjected to sunlight is shown in Fig. 8 which shows that the percentage of degradation of drug is photo stable as listed in Table-7.

Thermal degradation: Thermal degradation was performed by placing mefenamic acid in volumetric flask in an oven at

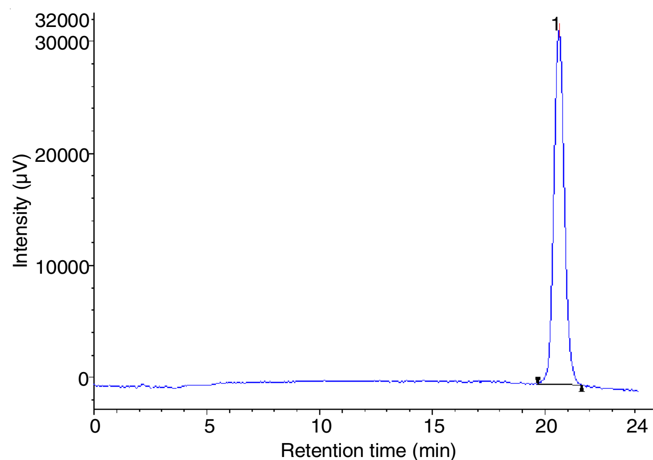

Fig. 8. Representative chromatogram of mefenamic acid on photolytic degradation

80 ºC for 4 days. Significant degradation (> 10%) is shown in Table-7 without any appearance of additional peak on chromatogram (Fig. 9).

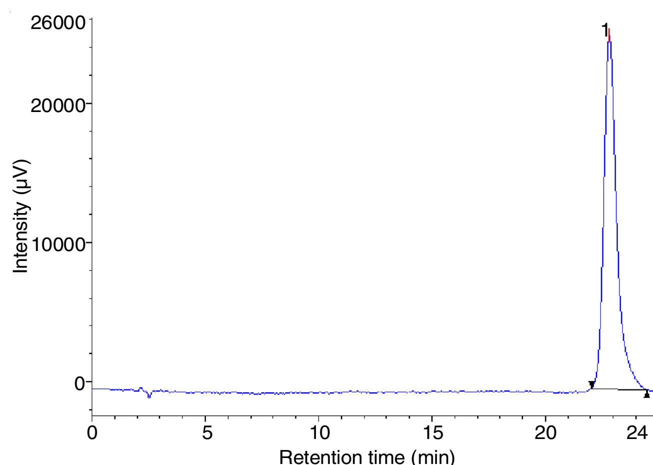

Fig. 9. Representative chromatogram of mefenamic acid on thermal degradation

Conclusion

The developed stability-indicating and validated RP-HPLC method is precise, accurate and robust, and can be applied for the determination of mefenamic acid in pharmaceutical dosage forms. The drug was found to be more degraded when exposed to oxidation stress conditions as it degraded by 37.44 % with the appearance of two degradants peaks and least degraded when exposed to hydrolysis (acidic, basic and neutral), thermal and photo-stress conditions.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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