

Development of Validated Analytical Method of Mefenamic Acid in an Emulgel (Topical Formulation)

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Abstract

An isocratic reversed phase HPLC method has been developed for determination of mefenamic acid on a Grace, alltima C18 column (250x4.6 mm) using a mobile phase consisting of methanol : ammonium acetate (pH 6) (67:33 v/v) at a flow rate of 1 mL/min. Detection was carried out at 254 nm. Retention time of mefenamic acid was 9.85 (± 0.36) mins. The method was validated with respect to specificity, linearity, accuracy, precision, ruggedness, and robustness.

The proposed method is simple, precise, sensitive, and reproducible and is applicable for quantification of mefenamic acid in an Emulgel (topical formulation) developed and formulated in our laboratory.

Keywords: Isocratic; Reverse phase; Mefenamic acid; Emulgel.

Introduction:

Mefenamic acid 2-[(2, 3-dimethylphenyl) amino] benzoic acid is a non-steroidal anti-inflammatory agent with analgesic, anti-inflammatory, and antipyretic properties¹. mefenamic acid is official in IP², BP³, USP⁴ and EP⁵. Literature survey reveals the availability of several analytical methods being developed and validated for mefenamic acid alone or in combination with other drugs. Various methods have been developed either for bulk or in oral dosage form⁶⁻¹¹. But no analytical method has been reported for estimation of mefenamic acid in topical formulation (Emulgel).The present study describes a validated isocratic RP-HPLC method for mefenamic acid in an Emulgel (topical formulation) developed and formulated in our laboratory.

Materials And Methods:

Apparatus:

Chromatography was performed on Jasco Binary system with, two PU2080 PLUS intelligent HPLC pumps, UV2075 PLUS intelligent UV detector, Solvent Mixing module MX-2080-31, Rheodyne[®] manual injector system, LCNet II / ADC system interface and Borwin[®] Chromatography Software, Jasco Corporation, Japan and 5 μ m Grace Alltima C18 column having dimensions of 250 x 4.6 mm id was used.

Materials and Reagents:

Mefenamic acid standard was obtained as a gift sample from Flamingo Pharmaceuticals Ltd (Navi Mumbai, India). HPLC grade methanols, water, ammonium acetate used were purchased from S.D. Fine Chemicals (Mumbai, India).

Chromatographic Conditions:

Chromatographic separation was carried out on a Grace, Alltima, 250mmx4.6mm, 5 μ m C-18 reverse phase column. Mobile phase consisting of methanol and ammonium acetate (pH-6) was pumped at a flow rate of 1 mL/min. The elution was monitored at 254 nm and the injection volume was 20 μ L. Validation of the method was performed following the International Conference on Harmonization (ICH) guidelines¹².

Preparation of the Mobile Phase and Standard Solutions:

Methanol and ammonium acetate used for the mobile phase were filtered through a 0.45 μ m membrane filter (Ultipore N -66R Nylon 66; Pall Corp.,) and degassed by ultrasonication for 15 min.

The standard Stock solution was prepared by dissolving mefenamic acid in methanol separately to get a solution containing 1 mg /mL for mefenamic acid. The working standard solution of mefenamic acid was prepared by diluting 0.2 mL stock solution to 10 mL with methanol to obtain a solution containing 20 μ g /mL mefenamic acid.

Preparation of the Sample Solutions:

1 g of emulgel was accurately weighed in 10 mL volumetric flask. About 6 mL of methanol was added, further was then vortexed and sonicated for 15 minutes with intermittent swirling to get uniform dispersion. Volume was made up with methanol to obtain Sample Stock Solution of concentration 1000 μ g/mL. This stock solution

was filtered through 0.45 μm PTFE filter. The working sample solution was prepared by diluting 0.2 mL stock solution to 10mL with methanol to obtain a solution containing 20/ μg mL mefenamic acid.

Analysis of developed Formulation:

Assay of developed topical formulation containing mefenamic acid (1%w/w) was performed by preparing the sample solutions as described earlier in the preparation of the sample. Six injections of above prepared sample and standard solutions were injected. The assay of the developed formulation sample was calculated by comparing the areas of standard and sample peaks.

Validation of the Method:

Calibration curve (linearity of the HPLC method):

The calibration curve was constructed by plotting concentrations of mefenamic acid versus peak areas, and the regression equations were calculated. The linearity of the method was investigated by using concentrations of 5, 10, 20, 30, 40, 50, 60/ μg /mL. These concentrations were prepared by diluting appropriate volumes of working standard with methanol. The retention time of mefenamic acid was 9.85(\pm 0.36) mins.

System suitability study:

For this study, 20 μL blank solution [methanol] was injected and run for 12 mins. After this, 20 μL standard solutions in six replicates were injected, and the RSD of the resultant peak areas was calculated.

Accuracy (recovery):

Accuracy of the method was studied by recovery experiments using the standard addition method at three different levels (80, 100, and 120%). Known amounts of standard solutions containing mefenamic acid (8, 10, and 12 μg) were added to prequantified sample solutions to reach the 80, 100, and 120% levels. These samples were analyzed and recovery was calculated.

Precision (repeatability):

Precision of the assay method was demonstrated by injecting six different sample solutions containing mefenamic acid equivalent to 20/ μg /mL and RSD of the mean assay value was calculated.

Intermediate precision (ruggedness):

Intermediate precision of the method was demonstrated by carrying out the experiment on different day, by different analyst, and using different C18 column.

Robustness:

Robustness of the method was demonstrated by deliberately changing the chromatographic conditions. The flow rate of the mobile phase was changed from 1.0 to 0.9 mL/min and from 1.0 to 1.1 mL/min. The composition of mobile phase was changed from 67:33 to 70.3:33 and from 67:33 to 64.35:33. The sample solutions described for the robustness study were applied onto the column in triplicate, and the responses were determined.

Limit of Detection (LOD) and Limit of Quantification (LOQ):

LOD and LOQ of mefenamic acid was calculated using the following equations as per International Conference on Harmonization (ICH) guidelines.

$$\text{LOD} = 3.3 \times \sigma/S, \text{LOQ} = 10 \times \sigma/S$$

Where, σ = Standard deviation of response S = Slope of regression equation.

Specificity:

Specificity of the method was demonstrated by injecting the blank solution, standard solution, sample solution, and solvent extracted placebo and the responses were determined.

Results and Discussions:

Several mobile phase compositions were tried. A satisfactory separation and good peak symmetry were obtained by using the described methanol: ammonium acetate

(pH-6)(67:33v/v). Quantification was achieved with UV detection at 254 nm. Representative chromatograms of standard mefenamic acid and mefenamic acid in Emulgel (topical formulation) are shown in **Figure 1** and **Figure 2** respectively.

System suitability tests were carried out on freshly prepared standard solutions ($n = 6$) containing mefenamic acid. System suitability parameters obtained with 20 μL injection volumes are summarized in **Table 1**.

Linearity regression data are summarized in **Table 2**, which shows A good linear relationship between concentration of mefenamic acid and peak areas was obtained over a concentration range of 5–60 μg /mL **Figure 3**.

The correlation coefficient (r^2) was found to be 0.9996 for mefenamic acid which ensures that a good correlation existed between the peak area and analyte concentration. The LOD was found to be 1.34 μ g/mL for mefenamic acid. LOQ was found to be 4.06 μ g/mL. These values indicate that the method is sensitive.

In the precision studies, RSD of mean assay values was found to be 1.62% for mefenamic acid. These %RSD values which are well below 2% indicate that the repeatability of this method is satisfactory. Thus there exists a closeness of agreement in repeated measurements of peak response. The intermediate precision study revealed that the method is rugged with %RSD values of 1.96, 1.10 and 0.79 for performing the analysis by changing the column and analyst and performing the analysis on another day respectively. As evident the RSD values of the data obtained are well below 2% indicating that method can be repeated successfully on different day, on different column and by different analyst.

Accuracy studies indicated that the percent recoveries were obtained from the difference between the areas of spiked and unspiked samples. The mean recovery of the added standard drug was 98.80%. This means recovery value is well within the range of 98-100%, indicating the method is accurate.

Specificity studies indicated good resolution was obtained between the drugs and excipients showing complete separation of mefenamic acid. No interference from excipients, impurities, or degradation products ensured that the peak response was due to mefenamic acid only. The resolution between excipient peak and mefenamic acid peak was found to be 3.10.

Robustness studies signified that the results of the method remained unaffected by small, deliberate changes in the flow rate and mobile phase composition. The RSD of mean assay values was found to be 0.87 % with a flow rate of 0.9 mL/min. The RSD of mean assay values was found to be 0.80% with a flow rate of 1.1 mL/min. Also, RSD of mean assay values was found to be 1.27% at mobile phase composition of 70.3:33v/v and 1.28% at mobile phase composition of 64.3:33 v/v. The RSD values of the data obtained are well below 2% indicating that method is robust i.e. it is reliable during normal use. All the validation data are summarized in **Table 3**.

The assay results obtained by using the proposed method for the analysis of developed Emulgel formulation containing mefenamic acid (1% w/w) were in good agreements with the labeled amounts of mefenamic acid. The average contents of mefenamic acid in developed formulation were 0.009 gm/1g (0.009% w/w).

Conclusions:

The proposed RP-HPLC method is accurate, precise, sensitive, selective, and rapid for the determination of mefenamic acid in an emulgel (topical formulation) developed and formulated in our laboratory.

Acknowledgment:

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Figures legends

Fig. 1: Chromatogram of Standard Mefenamic Acid (R.T. 9.85 (\pm 0.36)) at 254 nm.

Fig. 2: Chromatogram of Mefenamic Acid in Topical Formulation (R.T. 9.9 (\pm 1.10)) at 254 nm

Fig. 3: Calibration Curve Diagram For Mefenamic Acid

Fig. 1: Chromatogram of Standard Mefenamic Acid (R.T. 9.85 (±0.36)) at 254 nm.

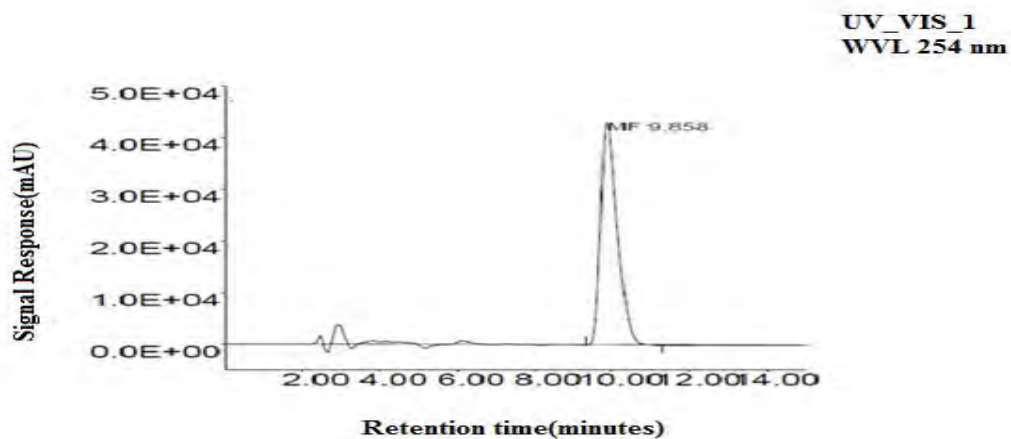


Figure 2: Chromatogram of Mefenamic Acid in Topical Formulation (R.T. 9.9 (±1.10)) at 254 nm

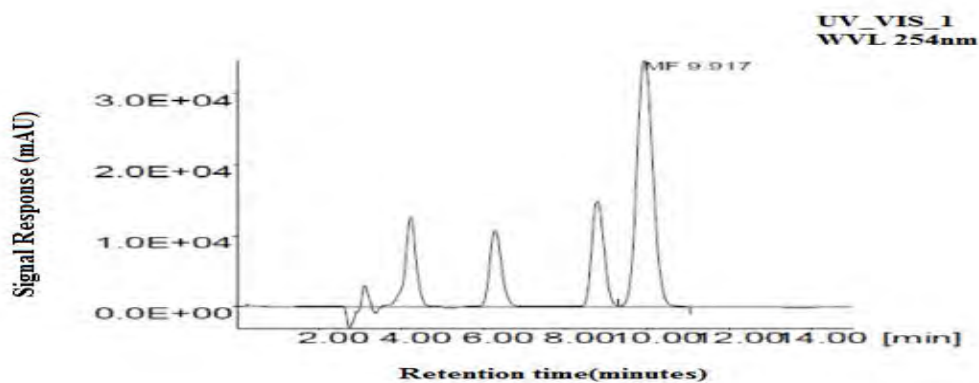
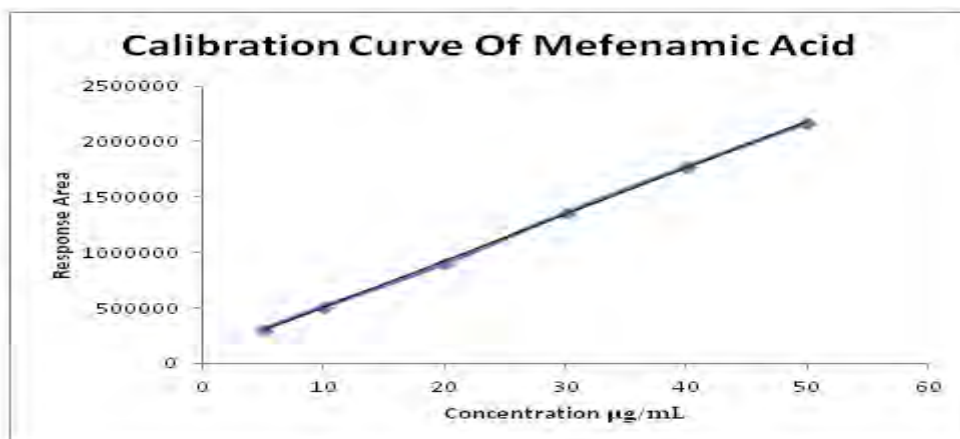


Fig. 3: Calibration Curve Diagram For Mefenamic Acid

$$y=41804+88511x$$

$$R^2 = 0.9996$$



Tables

Table 1. System Suitability Test Parameters For Mefenamic Acid.

Parameter	Mefenamic Acid
Retention Time(min)	9.82 ±1.14
Theoretical plates	3284.31± 1.48
Asymmetry	1.617± 1.5
Area of response	1228147.51±1.56

Table2: Regression Analysis Of Calibration Curve For Mefenamic Acid

Parameter	Mefenamic Acid
Linearity range($\mu\text{g/mL}$)	5-60
Regression equation	$y = 41804x + 88511$
Correlation coefficient (R^2)	0.9996
Slope	41804
X-intercept	-2.117
Y-intercept	88511

Table 3: Summary Of Validation Parameters For The Proposed HPLC Method of Mefenamic Acid.

Parameter	Mefenamic acid
LOD ($\mu\text{g/ml}$)	1.34
LOQ ($\mu\text{g/ml}$)	4.06 $\mu\text{g/mL}$
Accuracy(% recovery)	98.8 \pm 0.60
Precision	95.43 \pm 1.62 ^a
Ruggedness	
Column change	102.41 \pm 1.96 ^a
Analyst change	95.66 \pm 1.10 ^a
Day change	93.93 \pm 0.79 ^a
Robustness (70.3:33v/v Mobile phase comp.)	104.46 \pm 1.27 ^b
Robustness (64.3:33v/v Mobile phase comp.)	98.17 \pm 1.28 ^b
Robustness (0.9mL/min Flow rate)	98.37 \pm 0.87 ^b
Robustness (1.1mL/min Flow rate)	98.51 \pm 0.80 ^b

^a mean assay values of 6 replicates

^b mean assay values of 3 replicates