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Original Article

DETERMINATION OF MEFENAMIC ACID IN A TOPICAL EMULGEL BY A VALIDATED HPLC METHOD

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ABSTRACT

Objective: The present study is aimed to develop and validate a simple, precise and accurate high-performance liquid chromatography (HPLC) method, according to ASEAN guideline for the validation of the analytical procedure, for the determination of mefenamic acid in a topical emulgel preparation.

Methods: An emulgel of 1 % mefenamic acid was prepared using carbopol 940 as a gelling agent and cremophor EL as an emulsifying agent. It was diluted with ethanol to make a sample concentration of 200 µg/ml. The method used a C18 column (5 µm; 250 x 4.6 mm) with the mobile phase, consisting of acetonitrile, acetic acid, and water in a ratio of 75:1:24. The column was maintained at 25 °C. The flow rate was 1 ml/min and the injection volume was 10 µl. The peak response was monitored by UV at 282 nm. It was validated for specificity, range, linearity, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ). In addition, forced degradation (hydrolysis, oxidation and dry heat) was performed to determine the capability of the proposed method to analyze the chemical stability of the drug samples during storage.

Results: The method was specific to the drug while other excipients did not interfere with the quantitation of mefenamic acid. It was linear in the concentration range of 1.29 to 806 µg/ml. LOD and LOQ were 4.88 and 14.78 µg/ml, respectively. Accuracy of the method was demonstrated by recovery experiments on the synthetic mixture method and the mean percent recovery was 101.10±1.56. Repeatability and intermediate precision were rugged with %RSD values of 1.30 and 1.07, respectively. The method could separate mefenamic acid from other degradation products of forced degradation.

Conclusion: The HPLC method presented herein is simple, accurate, sensitive and reproducible for the determination of mefenamic acid in an emulgel. It is served as a stability-indicating method and can be used for the analysis of the drug during product development and stability studies.

Keywords: ASEAN guideline, Emulgel, HPLC, Mefenamic acid, Stability-indicating method

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INTRODUCTION

Mefenamic acid or 2-(2,3-dimethyl phenyl) aminobenzoic acid (fig. 1) is a nonsteroidal anti-inflammatory drug (NSAID). It is often used for the treatment of mild to moderate pain, including headache, fever, dysmenorrhea, osteoarthritis, rheumatoid arthritis and inflammation [1]. The drug is classified as a biopharmaceutical classification system (BCS) class II that has low water solubility but high permeability [2]. It is conventionally available in the form of a tablet, capsule, and suspension for oral administration. The absolute bioavailability of the drug is about 90–100 % by which the dissolution is the critical process in drug absorption [3].

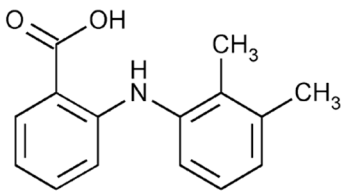

Fig. 1: Chemical structure of mefenamic acid

Topical preparation is another potential formulation that is used for the localized effect at the site of its application. Emulgel is a promising topical preparation for the delivery of hydrophobic drugs such as mefenamic acid, diclofenac, ketoprofen and ketoconazole [4-6]. It is an emulsion that is gelled by gelling agents. This preparation has many advantages; for example delivery of poorly soluble drugs at a specific site, avoidance of gastrointestinal incompatibility and more patient compliance. Several attributes of emulgel are characterized to ensure the quality and consistency of drug products. UV spectrophotometry and high-performance liquid chromatography (HPLC) are often used to analyze the content of mefenamic acid in raw material, tablet dosage form and human plasma [7-9]. Binhashim and Hammami developed an HPLC method to determine mefenamic acid in human plasma [10]. They used diclofenac as an internal standard and the drugs were separated on a C18 column with a mobile phase of 0.025 M phosphate buffer pH 6.0 and acetonitrile (65:35). This method was accurate and suitable for use in stability study because no interference was co-eluted with mefenamic acid and the limit of quantification (LOQ) of the drug was acceptable (0.05 µg/ml). Kumar *et al.* simultaneously determined mefenamic acid, dicyclomine HCl and pamabrom in a tablet. They used a C18 column as a stationary phase and a mixture of 20 mm potassium dihydrogen orthophosphate pH 5.9 and acetonitrile (30:70) as a mobile phase. The method was followed ICH guideline and the drug was detected at 285 nm, showing the linear range in the concentration of 0.5-15 µg/ml. It was an accurate procedure with a mean recovery of 99.73±0.68 %. However, a few studies have developed a UV spectrophotometry to analyze mefenamic acid in emulgel. They dissolved a quantity of gel, containing hydroxypropylmethylcellulose and sodium carboxymethylcellulose as gelling agents, in methanol and the drug content and drug release were determined at 285 nm [11, 12]. This method showed linearity over a concentration of 5-30 µg/ml and it was useful for evaluation of the drug in this topical formulation. It has been reported that mefenamic acid is not a stable drug and its degradation products could increase undesirable effects [13, 14]. However, there is limited information on the stability-indicating HPLC method used to determine the drug content in the emulgel during stability study [15]. This study was aimed to develop and validate an HPLC method to analyze mefenamic acid in an emuslgel, according to ASEAN guideline for the validation of the analytical procedure. Forced

degradation studies in hydrolysis, oxidation and thermal degradation were also performed to determine the stability-indicating capability of the method.

MATERIALS AND METHODS

Chemicals and reagents

Mefenamic acid was purchased from P. C. drug center Co. Ltd, Thailand and its reference standard were obtained from Sigma-Aldrich, USA. Cremophor EL was from BASF Corp., USA. Carbopol 940, isopropyl myristate, methylparaben, and propylparaben were purchased from P. C .drug center Co .Ltd, Thailand .Acetonitrile was HPLC grade from RCI Labscan, Thailand.

Preparation of 1% mefenamic acid emulgel

Mefenamic acid (1 g) was dissolved in 2.5 ml of ethanol and the mixture was prepared as the water phase by adding an aliquot of water and 0.5 % paraben concentrate. The oil phase was obtained by mixing 4 ml of cremophor EL and 2 ml of isopropyl myristate. Both phases were incubated at 70 °C before adding the oil phase into the water phase. The mixture was homogeneously mixed with the gel phase of 0.5 % carbopol 940 and the emulgel product was filled in an aluminum tube.

Preparation of sample solution

Emulgel sample (2 g) was weighed and transferred into a 50 ml volumetric flask. An aliquot of ethanol (25 ml) was used to dissolve the sample before sonication for 15 min. Ethanol was added to the mark and then a 2-fold dilution with ethanol was performed to make the sample solution (200 µg/ml). It was filtered through a 0.45 µm nylon membrane before injection.

Preparation of standard solution

Mefenamic acid reference standard (100 mg) was accurately weighed and dissolved with ethanol in a 50 ml volumetric flask. The stock solution of the standard was diluted with ethanol to make the working solutions of 20, 100, 200, 400 and 800 µg/ml. They were filtered through a 0.45 µm nylon membrane and transferred to 2 ml vials.

Chromatographic condition

Ultimate 3000 HPLC system (Dionex Corporation, USA) was used to determine the drug content by using Chromeleon 7 software to perform data collection and interpretation. Chromatographic separation was carried out on an Inertsil® ODS-3 C18 column at 25 °C (4.6 x 250 mm, GL Sciences, USA). The mobile phase was the mixture of acetonitrile, acetic acid, and water in a ratio of 75:1:24 and it was pumped at the flow rate of 1.0 ml/min. The injection volume was set at 10 µl and the peak response was monitored by UV at 282 nm. The method was validated according to ASEAN guideline for the validation of the analytical procedure (specificity, range, linearity, precision, accuracy, the limit of detection (LOD) and limit of quantification (LOQ) [16].

System suitability test

The system suitability test was carried out by injections of five replicates of the standard solution (200 µg/ml). General acceptance criteria were in the following; the relative standard deviation (RSD) of the peak response (≤ 2.0 %), a theoretical plate of the column (≥ 2000) and the tailing factor of the peak (≤ 2.0).

Specificity

Chromatograms of standard solution, sample solution, gel base solution, and standard-spiked sample solution were used to compare the specificity of the method. It must separate the mefenamic acid drug from the other chemicals in the emulgel sample.

Linearity, the limit of detection (LOD) and limit of quantification (LOQ)

Mefenamic acid reference standard (100 mg) was accurately weighed in triplicate to prepare stock solutions. It was dissolved with ethanol and adjusted the volume to 50 ml. The stock solution was diluted with ethanol to make the standard solution at a concentration of 1.28, 3.20, 8.00, 20.0, 100, 200, 400 and 800 µg/ml. Standard calibration curves were constructed by plotting the peak response and the corresponding concentration. Linear regression analysis was used to determine the linearity. LOD and LOQ were calculated by multiplying factors of 3.3 and 10 to a ratio of the standard deviation of the response and the slope of the curve, respectively.

Accuracy

The accuracy of the proposed method was assessed by making a synthetic mixture. The mefenamic acid standard at three levels (80 %, 100 %, and 120 %) in the emulgel was prepared in the same manner as the sample solution (160, 200 and 240 µg/ml, respectively). It was performed in triplicate and ethanol was used to adjust the volume. These sample solutions were filtered through a 0.45 µm membrane filter before analysis. Percent recovery and its standard deviation (SD) were calculated to determine the accuracy [17].

Precision

Repeatability of the proposed method was determined by injecting six replicates of the sample solutions in the same day. Intermediate precision was assessed by assaying the sample solutions on another day. The precision was presented by the percent relative standard deviation (%RSD) of the peak response [17].

Forced degradation studies

Hydrolytic degradation studies

Mefenamic acid emulgel (2 g) was dissolved in 30 ml of ethanol. The mixture was transferred into a 50 ml volumetric flask and then sonicated for 15 min. Acid and basic hydrolysis were carried out in triplicate by transferring 5 ml of 1 M HCl or 1 M NaOH into the samples. They were refluxed at 80 °C for 1 h and subsequently, 5 ml of 1.0 N NaOH or 1.0 N HCl was added to neutralize the acid and basic samples, respectively. Ethanol was used to adjust the volume of 50 ml. The same procedure with distilled water was performed as neutral hydrolysis. The samples were diluted with ethanol to make the sample solutions (200 µg/ml) and filtered through a 0.45 µm membrane filter before analysis [18, 19].

Thermal degradation studies

Thermal degradation was assessed by putting the mefenamic acid emulgel in a hot air oven at 80 °C for 1 w. They were performed in triplicate. Samples (2 g) were transferred into a 50 ml volumetric flask. They were dissolved in ethanol and then sonicated for 15 min. The sample solutions were 2-fold diluted with ethanol and filtered into 2 ml vials.

Oxidative degradation studies

Oxidative studies were performed in triplicate by pipetting an aliquot of 30 % H_2O_2 solution into the sample stock solutions (400 µg/ml) to make a final concentration of 1.0 % H_2O_2. They were incubated in the dark at room temperature for 1 h. A 2-fold dilution with ethanol was prepared to make the sample solutions (200 µg/ml) and they were filtered in the vials before injection.

RESULTS AND DISCUSSION

System suitability test

It is used to determine the suitability of the chromatographic system to assure the reliable result of drug analysis. It was shown that %RSD of the peak response was 0.16 and peak asymmetry of mefenamic acid was 1.25±0.02. The number of theoretical plates was 12664±23.11. It met the general requirement of system suitability.

Specificity study

HPLC chromatograms showed that mefenamic acid was eluted as a sharp peak at 7.64 min and there were no peaks of other chemicals in the emulgel preparation that interfered with the drug peak (fig. 2A-2C). It was a rapid HPLC method when compared to other

studies [20, 21]. The chromatogram of the standard-spiked sample solution also showed a large peak at the same retention time (fig. 2D). These indicated that the proposed method was specific for the determination of mefenamic acid in the emulgel.

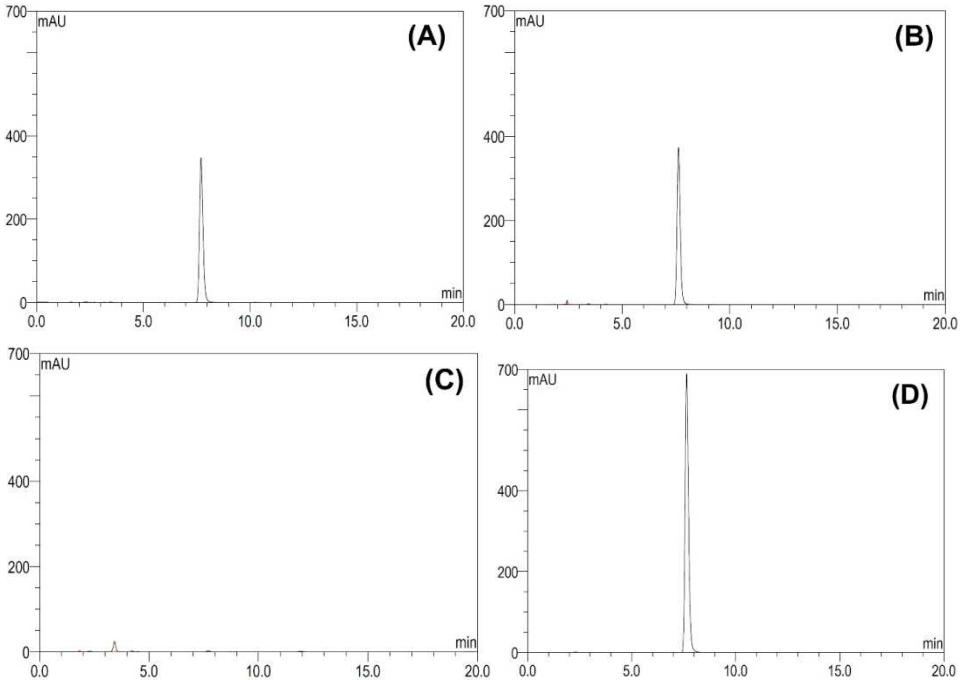

Fig. 2: Chromatograms of (A) standard solution of mefenamic acid (B) sample solution of mefenamic acid emulgel (C) solution of gel base and (D) standard-spiked sample solution

Linearity, LOD, and LOQ studies

Calibration curves of standard solutions were constructed and the results of regression analysis were summarized in table 1. It was shown that the curves were linear in the concentration range of 1.29-806 µg/ml with the correlation coefficient (r^2) of 0.9999. LOD and LOQ of the method were 4.88 and 14.78 µg/ml, respectively. It represented that the proposed method was more sensitive and useful for drug analysis in the emulgel preparation.

Accuracy study

The accuracy of the proposed method was determined by the synthetic mixture procedure. Different concentrations of mefenamic acid standard in the emulgel (80 %, 100 % and 120 % of test concentration) were prepared. Percent drug recovery was found in the range of 99.42±1.16 to 102.43±1.41 by which the mean percent recovery was 101.10±1.56 (table 2). They were in the acceptance criteria in the range of 97–103 % [17]. It indicated that the method was more accurate for drug analysis in the routine work.

Table 1: **Regression analysis of calibration curves**

Parameters	Results
Linearity range	1.29-806 µg/ml
Slope	0.3281
y-intercept	0.2064
SD of y-intercept	0.4848
Correlation coefficient	0.9999
LOD	4.88 µg/ml
LOQ	14.78 µg/ml

mean, n = 3.

Table 2: Accuracy of the method for analysis of mefenamic acid in drug products

Level	Concentration added (µg/ml)	Concentration measured (µg/ml)±SD	%Recovery±SD
80 %	160	163.99±2.26	102.43±1.41
100 %	200	198.85±2.33	99.42±1.16
120 %	240	243.34±2.26	101.39±0.94
Overall			101.10±1.56

mean±SD, n = 3.

Precision study

The precision of the method was assessed by several injections of the sample solutions. The results of repeatability showed that %RSD of the response were 1.30 and 0.90 in the first and second day, respectively (table 3). Moreover, the intermediate precision of all sample injections was 1.07.

They were within the generally acceptable criteria (≤ 2.7) [17]. Therefore, the proposed method was more precise.

Table 3: Precision of the method for analysis of mefenamic acid in drug products (n = 6)

Sample	Day 1		Day 2	
	Concentration measured (µg/ml)	%LA	Concentration measured (µg/ml)	%LA
1	201.75	100.87	198.48	99.24
2	196.83	98.36	198.53	99.29
3	202.16	101.23	196.34	98.16
4	199.42	99.81	201.00	100.47
5	199.57	99.98	200.60	100.25
6	196.52	98.02	200.69	100.36
Average	199.37	99.71	199.28	99.63
Repeatability (%RSD)	1.30		0.90	
Overall average	99.67			
Intermediate precision (%RSD)	1.07			

mean, n = 6.

Forced degradation studies

The proposed method was used to assess its capability to distinguish mefenamic acid in the emulgel from its degradation products for further use in the stability study. The drugs were degraded in the acid and basic-catalyzed hydrolysis by which the percent drug contents were 90.45±1.22 and 93.15±1.63, respectively (table 4). The drug was more stable in neutral hydrolysis with the drug content of 100.61±1.73 (fig. 3A-3C). Furthermore, the drug content was 94.67±1.74 under the oxidation with 1.0 % H_2O_2 (fig. 3D). These were consistent with other studies that demonstrated that mefenamic acid was decomposed under the acid and basic hydrolysis and oxidative reaction in which the degraded products were more polar and eluted faster than the parent drug [20, 22]. Previous studies also showed that the conditions of pH-catalyzed hydrolysis and H_2O_2 oxidation were the significant effects on the mefenamic acid degradation and the reaction kinetics was pseudo-first order [23]. However, the drug content was still in the general range of 90–110 %. These showed that the emulgel preparation made the mefenamic acid drug stable towards those conditions. However, the drug products were more sensitive to thermal degradation (80 °C for 1 w) and the drug content remained 76.21±1.41 (fig. 3E). The results were consistent with previous studies that mefenamic acid was susceptible to high temperature and thermal decomposition was nonspontaneous with Gibbs free energy (ΔG) of 131.01 kJ/mol [24]. These results presented that the proposed method was selective and could separate mefenamic acid from the other chemicals and degradation products. It serves as a stability-indicating method that uses for analysis of mefenamic acid in the emulgel preparation during stability studies.

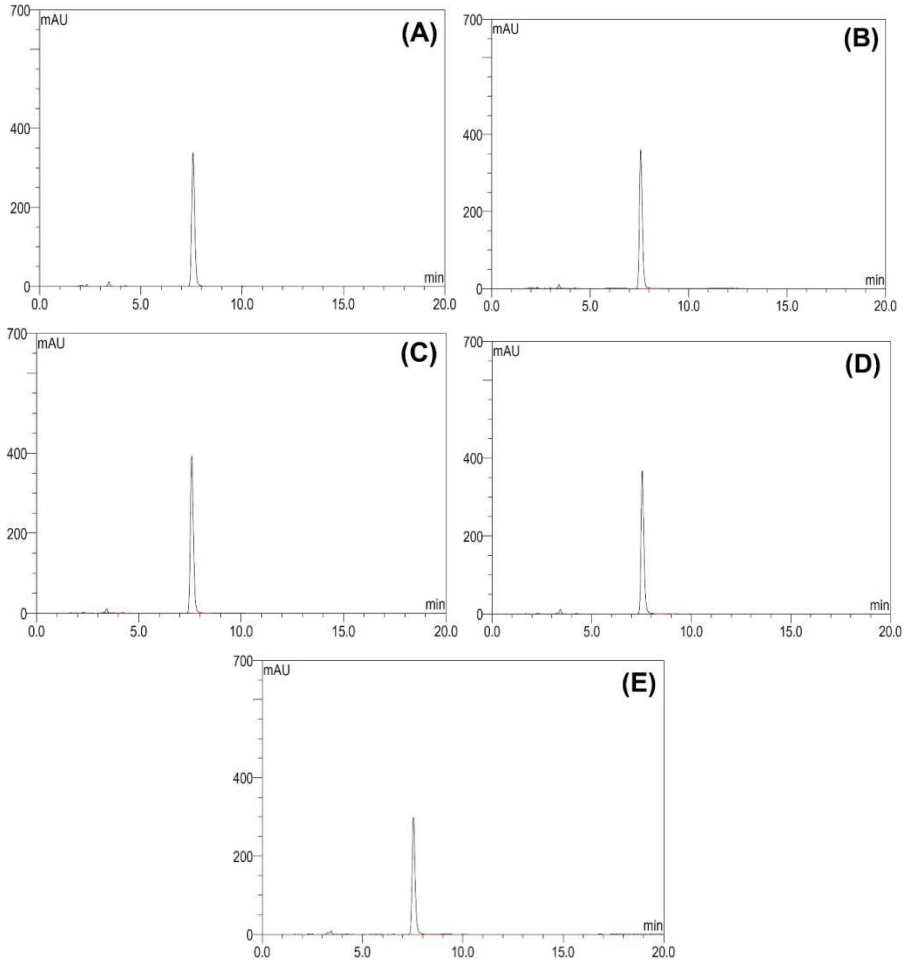

Fig. 3: Forced degradation studies of 1 % mefenamic acid emulgel. (A) acid hydrolysis, (B) basic hydrolysis, (C) neutral hydrolysis, (D) oxidative degradation and (E) thermal degradation

Table 4: Forced degradation studies of 1 % mefenamic acid emulgel

Conditions	%Drug content
Acid hydrolysis 0.1 N HCl, 80 °C/1 h	90.45±1.22
Basic hydrolysis 0.1 N NaOH, 80 °C/1 h	93.15±1.63
Neutral hydrolysis/Water, 80 °C/1 h	100.61±1.73
Oxidation/1.0%H_2O_2/1 h	94.67±1.74
Dry heat 80 °C/1 w	76.21±1.41

mean±SD, n = 3.

Application of the method for determination of mefenamic acid in a topical emulgel

The proposed method was used to analyze mefenamic acid in an emulgel preparation. It revealed that the mean drug content was 100.77±1.42 % from 12 different samples. It was in the general acceptant criteria with the labeled amount of 90–110 %. Therefore, the method was more sensitive, precise and accurate to determine mefenamic acid drug in the emulgel.

CONCLUSION

The developed HPLC method was validated, according to ASEAN guidelines for the validation of analytical procedure for the determination of mefenamic acid in a topical emulgel. It was simple, precise and accurate. Moreover, the chromatographic method could separate the mefenamic acid peak from any degradation products under stress testing. It was served as a stability-indicating method. Therefore, the proposed method can be used for quality control of mefenamic acid in the emulgel and analysis of drug products during the stability study.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally

CONFLICT OF INTERESTS

Declare none

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