Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(1):565-570

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Development and validation of thin layer chromatography-densitometry method for analysis of mefenamic acid in tablet

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ABSTRACT

Mefenamic acid is routinely used as tablet dosage forms. Thin layer chromatography (TLC) promotes for higher separation efficiencies, shorter analysis time, lower amounts of mobile phase, and efficient data acquisition and processing. There are various analytical methods for their estimation of mefenamic acid but till date there is no TLC method for its analysis. The paper presents the development and validation of a new TLC method for analysis of mefenamic acid in tablet. Separation was performed on silica gel 60 F_{254} plates. The mobile phase is comprised of chloroform: methanol (9.0: 0.1, v: v). Densitometry evaluation of the separated zones was performed at 320 nm. The drug was satisfactorily resolved with RF values of 0.55 ± 0.03. The accuracy and reliability of the method was assessed by evaluation of linearity (50-300 µg/mL), precision intra-day and inter-day RSD values were always less than 2, accuracy (102.45 % ± 1.36% for Sample A and 100.28 % ± 1.90 % for Sample B) in accordance with ICH guidelines. The proposed method is new, accurate and precise. Therefore, it is suitable for determination of mefenamic acid in tablet for analytical and pharmaceutical purposes.

Keywords: mefenamic acid, thin layer chromatography, densitometry, validation

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INTRODUCTION

Mefenamic acid is a non-steroidal anti-inflammatory drug used to treat pain, including menstrual pain. It is typically prescribed for oral administration. Mefenamic acid has molecular formula $C_{15}H_{15}NO_2$ and molecular weight 241.29 g/mol. Chemically, mefenamic acid is 2-[(2,3-dimethyl phenyl) amino] benzoic acid as presented in Fig. 1. It is metabolized to 3-hydroxymethyl mefenamic acid and further oxidation to a 3-carboxy mefenamic acid may occur. The chemical properties for mefenamic acid are white to grayish-white microcrystalline powder, melting point 230 - 231 ^{o}C with effervescence, practically insoluble in water; soluble 1 in 185 mL of ethanol, 1 in 150 ml of chloroform, and 1 in 80 mL of ether; soluble in solutions of alkali hydroxides [1].

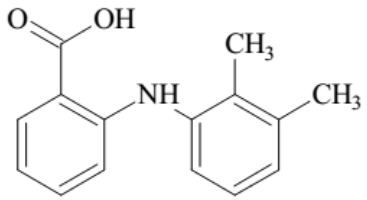

Fig. 1: Chemical structure of mefenamic acid

The assay of mefenamic acid in tablets is usually carried out by acidi-alkalimetry as Indonesian Pharmacopoeia [2]. Literature survey revealed that several methods were used to analysis of mefenamic acid in tablets. These methods include electrochemistry, high performance liquid chromatography, ultraviolet-visible spectrophotometry and atomic absorption spectrometry [3-6].

The aim of this study is performing very simple method in terms of mobile phase and program to analysis mefenamic acid in tablet, and validation of method in according to ICH guideline [7].

EXPERIMENTAL SECTION

Materials, chemicals and equipment

Mefenamic acid standards were obtained from PT Indofarma (Jakarta, Indonesia). Tablets of mefenamic acid were procured from retail pharmacies (Padang, Indonesia). Methanol, chloroform, glacial acetic acid and ether were obtained from E. Merck and of analytical grade. A Camag TLC system equipped with a sample applicator Namomat 4, twin trough plate development chamber, TLC Scanner III, Reprostar and Wincats 4.02, integration software (Switzerland). Pre-coated silica gel 60 F_{254} TLC aluminum plates were obtained from E. Merck, Jakarta (Indonesia).

Preparation of standard solution

Weigh accurately 25.3 mg mefenamic acid and was dissolved in 20 mL chloroform in 50 mL volumetric flask. This solution was sonicated for 15 minutes at 30 ^{o}C and then added chloroform up to mark to get the strength of 506 µg/mL mefenamic acid.

Method Development

Mefenamic acid solution was prepared using chloroform as solvent. The TLC plates were pre washed with methanol and activated by keeping at 115°C for about 30 minutes. Solutions of 2.0 µL were applied on the TLC plates as using Camag Nanomat 4. Application positions were at least 10 mm from the sides and 10 mm from the bottom of the plates. Mobile phase components were mixed prior to use and the development chamber was left to saturate with mobile phase vapor for 15 minutes before each run. Mobile phase components were listed in Table 1.

Table 1: Component of mobile phase used in TLC of mefenamic acid analysis

Component of mobile phase	Ratio	Rf
Methanol : Chloroform : Acetic acid	8 : 10 : 0.2	0.87
Methanol : Chloroform : Acetic acid	3 : 7 : 2 drops	0.85
Dichloromethane : Methanol : Acetic acid	4 : 1 : 2 drops	0,86
Dichloromethane : Methanol	2 : 3	0,78
Dichloromethane : Methanol	4 : 1	0,78
Dichloromethane	1	0,88
Chloroform	1	0,30
Chloroform : Methanol	9.9 : 0.1	0.55

Development of the plates was carried out by the ascending technique to a migration distance of 8 cm. The plates were dried by hair dryer.

Densitometry scanning was done in absorbance mode at 320 nm using a deuterium lamp. The slit dimensions were set at 6 x 0.30 mm, the scanning speed at 20 mm/s and data resolution at 100 m/step. Single wavelength detection was performed because we are dealing with main component analysis and not impurities determinations where scanning at the individual λ values would be preferred.

These conditions were transferred to the TLC system and the results were evaluated with the aim of achieving an optimum separation between spots ($Rs \geq 2$) and a migration of spots with Rf values between 0.32 and 0.55 in order to ensure separation reproducibility.

Method Validation

Linearity

A stock solution with 506 µg/mL of mefenamic acid was prepared in chloroform. The volume of 1, 2, 3, 4 and 5 mL of stock solution were introduced by measuring pipette into separate 10 mL volumetric flask and then diluted with chloroform up to the mark. These solutions contain 50.6, 101.2, 151.8, 202.4, 253.0 µg/mL of mefenamic acid. A volume of 5 µL of each solution was applied on the TLC plate. This was done in triplicate and repeated for three days. For each concentration, the applied spot were evenly distributed across the plate to minimize possible variation along the silica layer. The linearity was evaluated visually by looking at the calibration curve of mefenamic acid.

Precision

The repeatability and time-different intermediate precision were determined simultaneously. Intra-day assay precision was found by analysis of standard drug at three times on the same day. Inter-day assay precision was carried out using at three different days and percentage relative standard deviation (% RSD) was calculated. The RSD was found to be less than two for both intra-day and inter-day precision. Repeatability of sample application

was assessed by spotting 101.2, 202.4 and 253.0 µg/mL of mefenamic acid solution, and three times. From the peak areas, the percentage RSD was determined.

Accuracy
The accuracy of the method was assessed by determination of the recovery of the method at three different concentrations (40%, 80% and 120% concentration) by addition of known amount of standard to the placebo. Solutions were prepared in triplicate and analyzed. This procedure was repeated for three consecutive days. Calibration curves to estimate the concentration of drug per spot were measured daily on the same plates as the samples. The accuracy was determined and expressed as percentage recovery.

Analysis of tablet samples
The method was used for quantization of mefenamic acid procured from local pharmacy. For sample preparation, chloroform was used as solvent for extraction and dilution. Twenty tablets containing mefenamic acid were milled well and weighed accurately. Portions of powder equivalent to 10 mg of mefenamic acid was weighed accurately and introduced into a 50 mL volumetric flask. The mixture was diluted up to 50 mL volume with chloroform; mixed well and filtered through Whatmann filter paper No 41 to obtain the sample stock solution. Further 5 µL of the test solution was applied on the pre-coated silica gel 60 F_{254} plate and from the peak area obtained; the amount of mefenamic acid in tablet was calculated using the calibration graph.

For the determination of mefenamic acid, sample solutions were prepared in triplicate and analyzed according to the method procedure. Sample and standard solutions were spotted on the same plate.

Reproducibility
Reproducibility is assessed by means of an inter-laboratory trial. Reproducibility should be considered in case of the standardization of an analytical procedure, for instance, for inclusion of procedures in Pharmacopoeias. These data are not part of the marketing authorization dossier.

Repeatability
Repeatability should be assessed using a minimum of 9 determinations covering the specified range for the procedure (e.g., 3 concentrations /3 replicates each).

RESULTS AND DISCUSSION

During the stage of method development different mobile phases were tried and the mobile phase comprising of chloroform and methanol (9.9: 0.1, v: v) was confirmed. Component of mobile phases used in TLC of mefenamic acid analysis were listed in Table 1. This table showed that Rf value 0.55 was the best mobile phase for TLC of mefenamic acid analysis by using silica gel 60 F_{254} plate.

A good linear relationship was obtained over the concentration range 50-300 µg/mL with linear regression Y = 12.558 X + 414.1 and coefficient correlation of 0.997 (Fig. 2). The LOD was found to be 27.10 µg/mL. The LOQ was found to be 82.13 µg/mL. The repeatability showed excellent % RSD less than 2 % after six applications (Table 2 and Table 3).

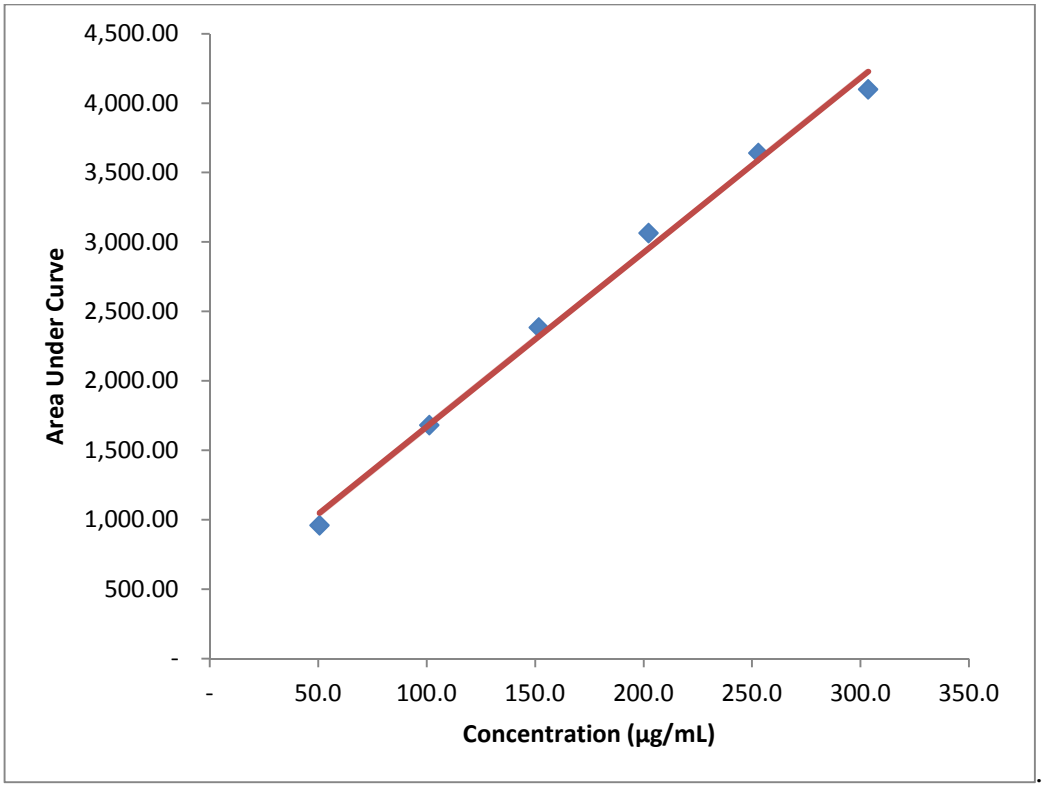

Fig. 2: Calibration curve of mefenamic acid

Table 2: Evaluation of interday precision of mefenamic acid

Concentration added (µg/mL)	Day	Area Under Curve	Concentration obtained (µg/mL)	SD	RSD
101.2	1	1681	100.9	1.59	1.43
		1675	100.4		
		1690	101.6		
	2	1710	103.2		
		1670	100.0		
		1701	102.5		
	3	1676	100.5		
		1640	97.6		
		1681	100.9		
202.4	1	2960	202.7	1.85	0.88
		2955	202.3		
		2945	201.5		
	2	2971	203.6		
		2962	202.9		
		2904	198.3		
	3	2910	198.7		
		2934	200.7		
		2951	202.0		
303.6	1	4250	305.5	1.06	0.35
		4221	303.1		
		4232	304.0		
	2	4244	305.0		
		4240	304.7		
		4235	304.3		
	3	4210	302.3		
		4216	302.7		
		4236	304.3		

Table 3: Evaluation of intraday precision of mefenamic acid

Concentration added (µg/mL)	Time on a day	Area Under Curve	Concentration obtained (µg/mL)	SD	RSD
101.2	1	1669	99.9	1.43	1.30
		1676	100.5		
		1688	101.4		
	2	1703	102.6		
		1675	100.4		
		1640	97.6		
	3	1677	100.6		
		1655	98.8		
		1675	100.4		
202.4	1	2958	202.6	0.70	0.33
		2947	201.7		
		2940	201.1		
	2	2970	203.5		
		2950	201.9		
		2948	201.8		
	3	2960	202.7		
		2952	202.1		
		2949	201.9		
303.6	1	4244	305.0	1.15	0.38
		4251	305.5		
		4250	305.5		
	2	4220	303.1		
		4235	304.3		
		4226	303.5		
	3	4215	302.7		
		4220	303.1		
		4217	302.8		

Recovery studies were carried out for estimation of the accuracy of the proposed method. These studies were carried out using standard addition method at three concentration levels. The obtained results were summarized in Table 4. The low RSD value (< 2) indicated the suitability of the method for routine analysis of mefenamic acid in pharmaceutical tablets.

Table 4: Standard addition method for the recovery studies

% Added	Concentration of standard added (µg/mL)	Area Under Curve	Concentration after standard addition (µg/mL)	Concentration before standard addition (µg/mL)	% Recovery	Average	SD	RSD
40	79.2398	3961.4	282.5	198.0995	106.48	105.93	0.51	0.48
		3955.2	282.0		105.86			
		3951.3	281.7		105.46			
80	158.4796	4905.6	357.7	198.0995	100.68	101.15	1.38	1.36
		4945.7	360.9		102.70			
		4893.2	356.7		100.06			
120	237.7194	6095.4	452.4	198.0995	106.98	105.48	1.31	1.24
		6034.2	447.5		104.93			
		6022.5	446.6		104.54			

The proposed chromatographic method was finally applied for the determination of mefenamic acid in the commercially available dosage forms. The obtained results of the present method were showed on Table 5. This confirms that the assay value lies within the limit specified in the Indonesian Pharmacopoeia [2].

CONCLUSION

In this work, TLC technique was developed and validated for the analysis of mefenamic acid in pharmaceutical tablets. The proposed method is simple, accurate and highly selective for mefenamic acid. The satisfactory sensitivity and simplicity make the methods suitable for routine analysis of mefenamic acid in quality control laboratories.

Table 5: Analysis of commercial tablets containing mefenamic acid

Sample	Labeled Content (mg/tablet)	Area Under Curve	Concentration obtained (µg/mL)	% Mefenamic Acid	Average	SD	RSD
A	500	2,975.3	203.9	101.85	102.45	1.36	1.33
		2,996.1	205.6	102.68			
		3,057.8	210.5	105.14			
		2,997.4	205.7	102.73			
		2,990.0	205.1	102.44			
		2,984.9	204.7	102.24			
		2,988.8	205.0	102.39			
		2,924.1	199.9	99.82			
		2,998.1	205.8	102.76			
B	500	2,971.7	203.7	101.71	100.28	1.90	1.90
		2,948.5	201.8	100.79			
		2,968.0	203.4	101.56			
		2,940.1	201.1	100.45			
		2,979.1	204.3	102.01			
		2,952.3	202.1	100.94			
		2,935.8	200.8	100.28			
		2,824.6	191.9	95.86			
		2,900.4	198.0	98.88			

Acknowledgements
The authors thank to Head of Central Laboratory, University of Andalas, Padang 25168, West Sumatra, Indonesia for facilities of this work.

REFERENCES

[1] AC Moffat, MD Osselton and B Widdop (Eds.). Clark's Analysis of Drugs and Poisons, Fourth Edition, Pharmaceutical Press, London, **2011**, 1617.
[2] Kementerian Kesehatan Repulik Indonesia. Farmakope Indonesia, Edisi 5, Kementerian Kesehatan RI, Jakarta, **2014**, 151-152.
[3] Riyanto and A Anshori. *Anal. Bioanal. Electrochem*, **2014**, 6(2), 159 – 169.
[4] FF Al-qaim, MP Abdullah, MR Othman and WM Khalik. *Int. J. Chem. Sci.*, **2014**, 12(1), 62-72.
[5] NA Alarfaj, SA Altamimi and LZ Almarshady. *Asian Journal of Chemistry*, **2009**, 21(1), 217-216.
[6] MY Khuhawar. TM Jehangir and FMA Rind. *Jour. Chem. Soc. Pak.*, **2001**, 32(4), 226-228.
[7] International Conference on Harmonization (ICH). *Validation of Analytical Procedures*, Text and Methodology, Q2 (R1), **2005**