



Analysis of Mefenamic Acid Residue in Urine of Herbal Medicine for Aches and Pains Consumers Using Ultraviolet-Visible Spectrophotometry Method

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ABSTRACT

Mefenamic acid is the chemical drug that has been added to herbal medicine. With the presence of these chemical additives that are not controlled will be harmful to society. So it is necessary to determine the presence of residual mefenamic acid content in the urine of consumption of herbal medicine aches and pains by ultraviolet-visible spectrophotometry. The sampling technique in this study used the Non-Probability Sampling technique with a total of 30 urine samples consuming stiff rheumatic herbs in Desa Panggang, Glagah, Lamongan. Based on the research that has been done, it was found that 30 urine samples of consuming herbal medicine A, B, C, and D were positive for the chemical mefenamic acid with the highest level of 7.16% and the lowest level of 2.17%. From the results of the analysis of the normality test and homogeneity test, it is obtained that the data are normally distributed and homogeneous. Based on the results of the Independent T Test, the value of Sig. (2-tailed) of 0.000 < 0.05, which means that there is a mefenamic acid content in the urine of those who consume herbal medicine aches and pains.

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INTRODUCTION

Traditional Indonesian medicine, also known as herbal medicine, is created from a variety of natural ingredients derived from the ancestral heritage of the Indonesian people, passed down from generation to generation. It is used to prevent and treat disease, maintain and restore health, and promote wellness and beauty. In an effort to ensure the safety of Indonesians consuming herbal medicine, the government has established regulations governing the safety of herbal medicine, outlined in Regulation of the Minister of Health of the Republic of Indonesia No. 007 of 2012 concerning the Registration of Traditional Medicines, which stipulates that traditional medicines in circulation must not contain chemical drugs. Long-term consumption of herbal medicine containing chemical drugs can pose health risks, such as stomach problems, kidney failure, liver problems, and even death (Sidoretno and Rz, 2018).

The most frequently abused chemical drugs in herbal medicine preparations are pain relievers such as paracetamol, methampirone, ibuprofen, and mefenamic acid (Yuliarti, 2010). Mefenamic acid is a non-steroidal anti-inflammatory drug or NSAID used to treat osteoarthritis, rheumatism, and pain (Fauziah, 2015). The uncontrolled addition of mefenamic acid to herbal medicine can be dangerous to the public. Mefenamic

acid can be identified not only in herbal medicine but also in the urine secretions of users of herbal medicine for aches and pains.

In this research, mefenamic acid residues were analyzed in the urine of people that consuming herbal medicine for rheumatism using ultraviolet-visible (UV-Vis) spectrophotometry. UV-Vis spectrophotometry offers several advantages: it can analyze samples qualitatively and quantitatively, is selective, the analysis process can be carried out quickly and precisely, can be used to determine very small quantities of test compounds, and produces fairly accurate data (Rohmah et al., 2021). Besides using UV-Vis spectrophotometry, BKO analysis in herbal medicine for rheumatism can be performed using thin-layer chromatography (TLC), as was done by (Gitawati, 2013) in her study entitled Analysis of Adulteration of Herbal Medicine for Rheumatism Obtained from Markets in Jakarta and its Surrounding Areas. The drawback of the TLC method is that it requires persistence and patience to obtain the desired spots.

Based on the description above, a question arises: whether mefenamic acid is present in the urine of people consuming herbal medicine for rheumatic pain using ultraviolet-visible spectrophotometry, and what the levels of mefenamic acid are in the urine of people consuming herbal medicine for rheumatic pain using ultraviolet-visible spectrophotometry. The purpose of this study was to determine the presence of the chemical compound mefenamic acid in the urine of people consuming herbal medicine for rheumatic pain using ultraviolet-visible spectrophotometry, and to determine the levels of the chemical compound mefenamic acid in the urine of people consuming herbal medicine for rheumatic pain using ultraviolet-visible spectrophotometry.

METHODOLOGY

The research design used descriptive design, using quantitative analysis of mefenamic acid levels in urine samples from users of herbal medicine for rheumatic pain using UV-VIS spectrophotometry. The samples used in this study were urine from residents of Panggang Village, Glagah District, Lamongan Regency who consumed herbal medicine for rheumatic pain. They had previously completed a consent form as study respondents. The instruments used were UV-VIS spectrophotometry for UV-1600 PC Spectrophotometer type, analytical balance (Fujitsu), volumetric flask, watch glass, funnel, stirrer, dropper, measuring cylinder, spatula, volumetric pipette, separating funnel, and burette. The materials used in this study were urine from users of herbal medicine for rheumatic pain, pure mefenamic acid, 100 µg/mL mefenamic acid stock solution, pure methanol, 70% alcohol, and distilled water. The sampling technique in this study uses a non-probability sampling technique, namely sampling carried out with the entire population being used as a research sample or the number of research samples being the same as the number of existing populations.

a. Preparation of 1000 µg/mL Mefenamic Acid

Stock Solution Weigh 100 mg of pure mefenamic acid powder and dissolve it in pure methanol in a 100 mL volumetric flask.

b. Preparation of 50 µg/mL Mefenamic Acid Standard Solution

Take 2.5 mL of 1000 µg/mL mefenamic acid stock solution, add pure methanol to the mark, and mix in a 50 mL volumetric flask.

c. Determination of the Maximum Wavelength of Mefenamic Acid

Pipette 2.5 mL of 50 µg/mL mefenamic acid stock solution and dissolve it in pure methanol in a 50 mL volumetric flask. The maximum absorbance is then measured at a wavelength of 200-400 nm using pure methanol as a blank.

d. Preparation of a Standard Curve for Mefenamic Acid

A standard solution of mefenamic acid (0.5; 1; 1.5; 2; 3; 5; 7; 9; 10) was prepared at µg/mL from a standard solution of 50 µg/mL. The reading was then taken using a UV-Vis spectrophotometer at the maximum wavelength.

e. Urine Sample Preparation

A 10 mL urine sample was taken, added with 10 mL of chloroform, placed in a separating funnel, and re-extracted twice. The urine extract (top layer) was then transferred to a petri dish and allowed to stand for 1 hour.

f. Determination of Mefenamic Acid Levels in Urine Samples

1 mL of the top layer of urine extract was pipetted and dissolved in a 10 mL flask with pure methanol. This was then measured using a UV-Vis spectrophotometer at the maximum wavelength.

g. Determination of Mefenamic Acid Levels in Herbal Medicine

Samples for Aches and Pains Each herbal medicine sample was weighed at 300 mg. It was then dissolved in methanol in a 50 mL volumetric flask. The solution was filtered, discarding the first 5 mL of filtrate. 1 mL of the filtrate was transferred to a 10 mL volumetric flask with pure methanol. The results were then measured using UV-Vis spectrophotometry in triplicate.

h. Precision Determination

Precision was determined by calculating the standard deviation (SD) of the absorbance value of a 5 µg/mL mefenamic acid solution at the maximum wavelength, 10x. Precision measurements were performed on the same day and on different days.

$$SD = \frac{\sqrt{\sum(x - \bar{x})^2}}{n-1}$$

$$\%RSD = \frac{SD}{\bar{x}} \times 100\%$$

i. Accuracy Determination

Accuracy in this study was determined by measuring the absorbance of three extracted urine samples.

- 1) 1 ml of the first urine extract was pipetted into three 10 ml volumetric flasks. Then, 0.5 ml, 2.8 ml, and 1.8 ml of standard mefenamic acid solution at concentrations of 1 µg/mL, 5 µg/mL, and 9 µg/mL were added to each flask, respectively. Each flask was then made up to the mark with pure methanol. The readings were then taken using a UV-Vis spectrophotometer at the maximum wavelength;
- 2) The second urine extract sample was pipetted 1 ml, put into 3 10 ml volumetric flasks, then to each volumetric flask was added sequentially standard solution of mefenamic acid with concentrations of 1 µg/mL, 5 µg/mL, and 9 µg/mL as much as 0.5 ml, 2.8 ml and 1.8 ml. Then each volumetric flask was filled with pure methanol to the limit mark. Then read on UV-Vis Spectrophotometry with maximum wavelength;
- 3) The third urine extract sample was pipetted into 10 ml of three flasks, then 0.5 ml, 2.8 ml, and 1.8 ml of standard mefenamic acid solution at concentrations of 1 µg/mL, 5 µg/mL, and 9 µg/mL were added sequentially to each flask. Each flask was then filled to the mark with pure methanol. The readings were then taken using a UV-Vis spectrophotometer at the maximum wavelength. % Accuracy = (Measured Concentration) / (Actual Concentration) x 100%

$$\%Accuracy = \frac{\text{Measured Concentration}}{\text{Actual Concentration}} \times 100\%$$

j. Determining the Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ are determined based on the standard deviation (SD) and slope. These determinations are obtained by measuring the absorbance of a pure methanol solution as a blank at the maximum wavelength with 9x.

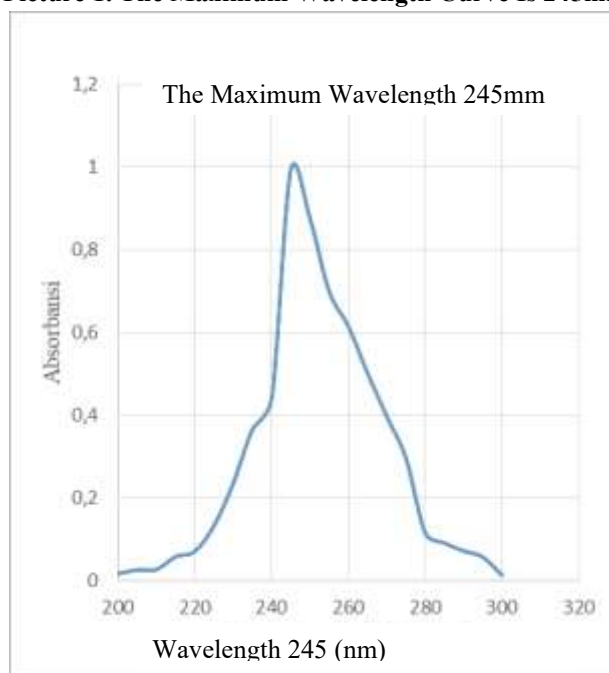
$$LOD = \frac{3 \times SD}{\text{Slope}(\lambda)}$$

$$LOQ = \frac{10 \times SD}{\text{Slope}(\lambda)}$$

RESULTS AND DISCUSSION

Determination of Maximum Wavelength

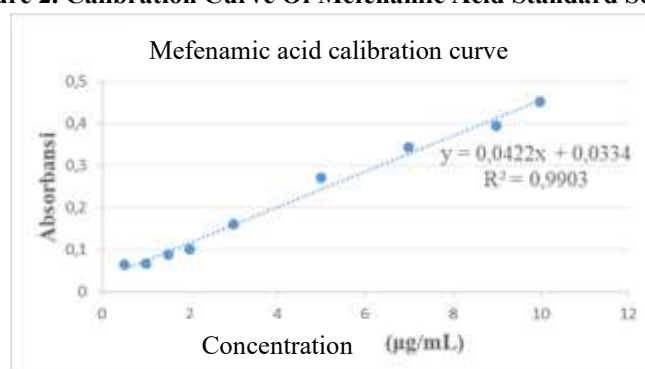
Figure 1 shows that the maximum wavelength is 245 nm. This maximum wavelength differs from other literature, which is 285 nm (Nerdy, 2007). This discrepancy is due to a shift in the absorption band of mefenamic acid caused by a blue shift that was named hypsochromic, which is a shift in the absorbance band toward a shorter wavelength accompanied by a decrease in absorption intensity.

Picture 1. The Maximum Wavelength Curve Is 245nm

The hypsochromic effect occurs due to differences or changes in the solvent used (Sayuti, 2017). This study used pure methanol, a volatile solvent, which makes the solution unstable when used.

Determining the Standard Curve for Mefenamic Acid

The standard curve is used to find a linear regression equation that can be used to determine the concentration of a substance whose absorbance has been measured. This linear regression equation represents the relationship between the series of mefenamic acid concentrations and the absorbance. The standard curve for mefenamic acid is based on absorbance values at concentrations of (0.5; 1; 1.5; 2; 3; 5; 7; 9; 10) $\mu\text{g/mL}$, at a maximum wavelength of 245 nm, and using methanol as a blank.

Picture 2. Calibration Curve Of Mefenamic Acid Standard Solution

From the data obtained the regression equation $y = 0.0422x + 0.0334$ with R^2 value = 0.9903. The y value is the response of the method, 0.0422 is the slope and 0.0334 is the intercept. The intercept is the intersection point on the line $y = 0$. The correlation coefficient (r) explains the linear relationship between the x-axis and the y-axis. The correlation coefficient value close to 1 indicates a linear relationship between the concentration and the resulting absorption. The criteria for accepting a good correlation coefficient according to (Sharger, 1985) is ≥ 0.9970 . The calculation of the mefenamic acid content in the sample will be obtained

from the regression equation where the y value is the absorbance produced in the sample and the x value is the mefenamic acid content in the sample.

Measuring Mefenamic Acid Levels in Urine

The next step is to determine the levels of mefenamic acid in urine samples from people consuming herbal medicine for aches and pains.

Table 1. Results Of Mefenamic Acid Cadre Measurements In Urine

No	Urine Sample (Code)	Absorbance		[Mefenamic Acid] (µg/ml)		Average Level (µg/ml)
		Test 1	Test 2	Test 1	Test 2	
1	1A	0,104	0,189	1,6730	3,6872	2,6801
2	2D	0,22	0,208	4,4218	4,1374	4,2796
3	3A	0,269	0,235	5,5829	4,7773	5,1801
4	4D	0,34	0,227	7,2654	4,5877	5,9265
5	5A	0,361	0,242	7,7630	4,9431	6,3531
6	6D	0,198	0,222	3,9005	4,4692	4,1848
7	7B	0,176	0,184	3,3791	3,5687	3,4739
8	8B	0,185	0,193	3,5924	3,7820	3,6872
9	9C	0,195	0,192	3,8294	3,7583	3,7938
10	10B	0,151	0,157	2,7867	2,9289	2,8578
11	11A	0,125	0,135	2,1706	2,4076	2,2891
12	12C	0,201	0,203	3,9716	4,0190	3,9953
13	13D	0,307	0,316	6,4834	6,6967	6,5900
14	14D	0,325	0,346	6,9100	7,4076	7,1588
15	15B	0,168	0,176	3,1896	3,3791	3,2844
16	16B	0,126	0,129	2,1943	2,2654	2,2299
17	17A	0,137	0,149	2,4550	2,7393	2,5972
18	18B	0,157	0,128	2,9289	2,2417	2,5853
19	19D	0,312	0,328	6,6019	6,9810	6,7915
20	20C	0,279	0,275	5,8199	5,7251	5,7725
21	21B	0,133	0,173	2,3602	3,3081	2,8341
22	22C	0,188	0,17	3,6635	3,2370	3,4502
23	23D	0,2	0,196	3,9479	3,8531	3,9005
24	24D	0,277	0,263	5,7725	5,4408	5,6066
25	25A	0,231	0,258	4,6825	5,3223	5,0024
26	26A	0,112	0,138	1,8626	2,4787	2,1706
27	27D	0,316	0,322	6,6967	6,8389	6,7678
28	28B	0,177	0,128	3,4028	2,2417	2,8223
29	29D	0,279	0,289	5,8199	6,0569	5,9384
30	30A	0,208	0,219	4,1374	4,3981	4,2678

Description:

A : Urine from person consuming herbal medicine A

B : Urine from person consuming herbal medicine B

C : Urine from person consuming herbal medicine C

D : Urine from person consuming herbal medicine D

The table shows that 30 urine samples from people consuming herbal medicine for aches and pains A, B, C, and D tested positive for the chemical drug mefenamic acid, with the highest concentration being 7.1588 µg/mL and the lowest concentration being 2.1706 µg/mL.

Measuring The Levels Of Mefenamic Acid In Herbal Madicine For Aches and pains

Table 2. Urine Results From Measuring The Levels Of Mefenamic Acid In Herbal Madicine

No	Herbal Madicine Sample	Absorbance			Concentration (µg/mL)			Average Concentration (µg/mL)
		1	2	3	1	2	3	
1	A	0,291	0,302	0,304	6,1043	6,3649	6,4123	6,2938
2	B	0,34	0,333	0,347	7,2654	7,0995	7,4313	7,2654
3	C	0,322	0,397	0,401	6,8389	8,6161	8,7109	8,0553
4	D	0,56	0,557	0,562	12,4787	12,4076	12,5261	12,4708

Description:

A : Herbal medicine sample A

B : Herbal medicine sample B

C : Herbal medicine sample C

D : Herbal medicine sample D

Herbal medicine sample A has an average concentration of 6.293 µg/mL. Herbal medicine sample B has 7.2654 µg/mL, herbal medicine sample C has 12.4708 µg/mL.

Precision Test

Table 3. Precision Test Results Data

Precision	Results %RSD	Required Standards
First Day	0,54	≤ 2%
Second Day	0,56	(Riyanto,2014)

The precision parameter indicates the degree of agreement between the test results measured through the distribution of results from the average repeatedly. Precision is measured as a standard deviation based on research conducted on sample replications taken from a homogeneous mixture (Harmita, 2004). The precision test results are obtained from equations (1) and (2). The calculation results in Table 3. obtained %RSD for the precision of the first day and the second day meet the criteria for a precise precision test, namely ≤ 2% (Riyanto, 2014). This shows that the level of accuracy of the method used is very good because it meets the test criteria.

Accuracy Test

Accuracy is expressed as the percent recovery (% recovery) of the added analyte. Accuracy is the degree of closeness between the test results and the procedure being validated and the true value. Accuracy test results are obtained from equation (3).

Table 4. Accuracy Test Results

Sample	% Recovery			Required Standards
	Concentration 1	Concentration 5	Concentration 6	
Urine 1	97,16	90,43	101,79	80 - 110% (Harmita, 2004)
Urine 2	100,00	105,97	100,42	
Urine 3	98,58	108,82	90,73	

The recovery (accuracy) of a compound in a matrix is acceptable if it is in the range of 80-110% of the actual concentration (Harmita, 2004). This indicates that the method provides good accuracy.

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

LOD and LOQ were carried out on pure methanol solution, the absorbance was read 9x.

Table 5. Test Results Data LOD and LOQ

Validation Parameters	Results
LOD	3,8416 µg/mL
LOQ	12,8054 µg/mL

From the calculation results in Table 5, the LOD value is 3.8416 µg/mL and the LOQ value is 12.8054 µg/mL. The LOD value shows that at a concentration of 3.8416 µg/mL is the lowest concentration of the mefenamic acid standard solution that can still be detected by UV-Vis spectrophotometry while the LOQ value shows that at a concentration of 12.8054 µg/mL is the highest concentration of the mefenamic acid solution that can still be quantified through a regression equation obtained from the mefenamic acid standard curve.

CONCLUSION

Based on the analysis of mefenamic acid residues in the urine of people consuming herbal medicine for aches and pains using UV-Vis spectrophotometry, the following conclusions were reached (1) thirty urine samples from people consuming herbal medicine for aches and pains obtained from respondents in Panggang Village, Glagah District, Lamongan Regency, tested positive for the chemical substance mefenamic acid; (2) Measurements of mefenamic acid levels in the urine of people consuming herbal medicine for aches and pains indicated that 30 urine samples from people consuming herbal medicine for aches and pains A, B, C, and D tested positive for the chemical substance mefenamic acid, with the highest concentration being 7.16% and the lowest being 2.17%. These four types of herbal medicine for aches and pains violate Indonesian Minister of Health Regulation No. 007 of 2012 concerning the prohibition of chemical substances in traditional medicines or herbal medicines.

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