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Analysis of Diclofenac Sodium in Traditional Medicine (Jamu) for Rheumatism in Banjarmasin using TLC-UV-Vis Spectrophotometric Method

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ABSTRACT

Jamu is one of Indonesia's most consumed traditional medicines. The government has clearly stated that traditional medicines are strictly prohibited from containing chemicals, both isolated and synthetic. Diclofenac sodium is one of the Non-Steroid Anti Inflammation Drug (NSAID) class drugs that have an effect as a pain reliever and anti-inflammatory, which is also often misused in traditional medicine preparations. The purpose of this study is to determine whether there is a chemical content of diclofenac sodium contained in the preparation of jamu pegal linu circulating in Banjarmasin using thin-layer chromatography (TLC) and to determine whether the validation of the method using a UV-Vis spectrophotometer meets the established requirements. The results of qualitative analysis using thin-layer chromatography showed that 4 out of 7 samples identified positively contained diclofenac sodium with an Rf value of 0.825. The results of quantitative testing using a UV-Vis spectrophotometer showed the acquisition of an r-value of 0.9996, %RSD of 0.97%, % recovery of 95.28%, 92.82%, and 95.48% for the addition of standard solutions of 10 ppm, 15 ppm, and 20 ppm, as well as LOD & LOQ values of 1.1625 ppm and 3.8750 ppm. Based on the analysis results, it can be stated that 4 out of 7 samples of jamu pegal linu circulating in the Banjarmasin area are positive for diclofenac sodium with levels ranging from 10.257 - 18.465 mg/g (1.026% - 1.846%).
Keywords: diclofenac sodium; jamu; traditional medicine; UV-Vis spectrophotometer

INTRODUCTION

Jamu is one of Indonesia's most consumed and used traditional medicines. Jamu is now becoming increasingly easy to find. Markets and roadside shops are one of the most common and easily found places to sell jamu. Jamu is traded and presented both in the form of powders and processed herbal decoctions ready to drink (jamu gendong) and is usually processed using generations of ancestral recipes in which its efficacy and safety for health have never been scientifically studied (Yuliarti, 2008).

The development of traditional medicine has increased the competition between traditional medicine and the pharmaceutical industry. The use of traditional medicine, which is believed to have minimal side effects, is one of the factors that cause the high demand for traditional medicine among the public. It creates demands for traditional medicine manufacturers to produce processed herbal products that can provide a quick reaction to a disease at a more affordable price. The existence of this demand does not make a few traditional medicine producers, especially traditional medicine, cheat by adding medicinal chemicals to their processed products.

The government has clearly stated that traditional medicines produced and distributed in the market are strictly prohibited from containing chemicals, both isolated and synthetic (BPOM, 2016). The results of BPOM supervision in 2021 showed that there were still 0.65% of traditional medicines on the market containing medicinal chemicals from a total of 9,915 samples of traditional medicine products in circulation. Although the resulting percentage is relatively small, this certainly does not rule out the possibility that many processed traditional medicine products in circulation contain medicinal chemicals. The use of medicinal chemicals in traditional medicinal preparations in the long term will cause various dangers to health and the body (BPOM, 2021)

Diclofenac sodium is a derivative of phenylacetic acid and is one of the Non-Steroid Anti Inflammation Drug (NSAID) or Non-Steroid Anti-Inflammatory Drugs (NSAIDs) which has an effect as a pain reliever and anti-inflammatory commonly used in patients with cases of rheumatoid arthritis and body aches. This drug is

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still often found and misused as one of the treatments in cases of body aches. The addition of diclofenac sodium in traditional medicine can cause various disorders of the gastrointestinal tract, ranging from nausea, vomiting, bloating, dyspepsia, and heartburn (BPOM, 2016).

Qualitative analysis of diclofenac sodium in traditional medicine for rheumatism in the Banjarmasin City area was carried out using the thin-layer chromatography (TLC) method. The choice of TLC as a qualitative test was chosen because in several previous studies conducted by Rosyada, et al, in 2019 diclofenac sodium can be detected using TLC. Quantitative analysis in this study using UV-Vis spectrophotometer. UV-Vis spectrophotometer was chosen because diclofenac sodium has chromophore and auxochrome groups that can be identified using UV-Vis spectrophotometer (Setyowati, et al*.,* 2021).

MATERIALS AND METHODS

Tools and materials

UV-Vis spectrophotometer (Shimadzu UV-1780), micropipette (Dragon Onemed®), volume pipette, ErlenmeyerErlenmeyer (pyrex®), volumetric flask (pyrex®), volume pipette (pyrex®), analytical balance, cuvette, chamber, GF254 silica plate (Merck®), capillary tube, funnel, and filter paper. Diclofenac sodium (BPFI), Methanol pro analysis (Merck®), silica GF 254 (Merck®), n-hexane (Merck®), ethyl acetate (Merck®), and 7 samples of traditional medicine products in Banjarmasin city.

Methods

Sample Preparation

A 1000 mg of traditional medicine samples were weighed, dissolved with 10 mL methanol ad, and then filtered (Nasution et al., 2022, with minor modifications).

Preparation of Diclofenac Sodium Standard Solution

A total of 50 mg of diclofenac sodium standard was weighed and put into a 50 mL volumetric flask, dissolved with methanol up to the limit mark, and obtained a standard solution concentration of 1000 ppm.

Qualitative Test with Thin-Layer Chromatography (TLC)

Silica gel GF 254 TLC plate was used. The mobile phase used was a mixture of ethyl acetate: n-hexane in a ratio of 7:3 (Rosyada & Yuanita, 2019). Spot appearance was carried out under ultraviolet (UV) light with a wavelength of 254 nm.

Method Validation

Preparation of Concentration Series of Diclofenac Sodium Standard Solution

From the 1000 ppm diclofenac sodium standard solution, a 100 ppm stock solution was made, which was then continued with the preparation of various concentration series starting from 10; 12.5; 15; 17.5; and 20 ppm.

Determination of maximum wavelength

The 10 ppm stock solution was measured in the 200-400 nm range to determine the maximum wavelength of diclofenac sodium.

Operating time

Diclofenac sodium 10 ppm stock solution was measured with the maximum wavelength obtained. Measurements were taken once a minute until the 10th minute when a constant absorbance measurement was obtained.

Linearity

Linearity measurements were made by measuring solutions of 10; 12.5; 15; 17.5; and 20 ppm for 3 replicate readings.

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

Concentration series solutions of 10; 12.5; 15; 17.5; and 20 ppm were measured for the absorbance at the maximum wavelength. LOD and LOQ were calculated by the calibration curve method with the equation**.**

$$LOD = \frac{3\,Sy/x}{S1} \qquad LOQ = \frac{10\,Sy/x}{S1}$$

Precision

The 10 ppm standard solution was measured for absorbance using the wavelength that had been obtained. Measurements were taken 10 times in the precision test treatment.

Accuracy

A standard solution of 100 ppm diclofenac sodium was taken as much as 0.5 mL; 0.75 mL, and 1 mL, then dissolved with 5 mL ad sample. Measure the absorbance of the solution at the maximum wavelength with 3x replication.

Quantitative Test Using UV-Vis Spectrophotometry

The sample of jamu pegal linu that has been prepared is then allowed to stand for operating time and then measured the absorption at the wavelength obtained using UV-Vis spectrophotometry. Absorbance measurements were carried out as many as 3x replications on each

sample. Then, the measurement results (absorbance) were entered into a linear regression equation to calculate the level of diclofenac sodium contained.

Data Analysis

Data analysis used in this study was qualitative and quantitative descriptive analysis. Qualitative descriptive analysis was used to describe the research results from laboratory tests, and the results in the form of numbers obtained were analyzed using Ms.Excel.

RESULTS

This qualitative test was carried out with 2 replicates with spot results that had similarities with the standard was spots A, B D and G (Figure 1).

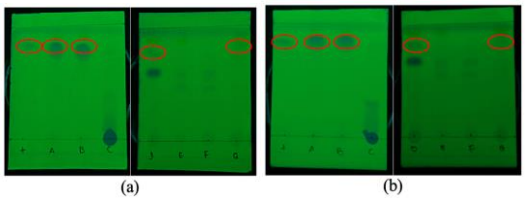

Figure 1. Qualitative test results using thin-layer chromatography

(a) Diclofenac sodium standard and sample A-G at wavelength 254 nm replicate 1; **(b)** Diclofenac sodium standard and sample A-G at wavelength 254 nm replicate 2.

Four of the seven samples of jamu pegal linu had R*f* values that were almost the same as the R*f* value of the standard comparison of diclofenac sodium, so it can be concluded that the four samples were positive for diclofenac sodium (Table I).

Maximum wavelength of diclofenac sodium obtained was 281 nm (Figure 2) and from the results of operating time measurements, a constant absorbance was obtained at minute 0 with an absorbance value of 0.392 nm (Figure 3).

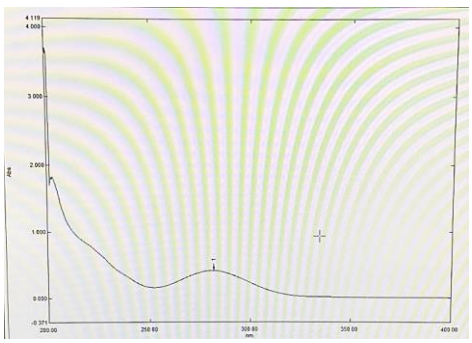

Figure 2. Peak of diclofenac sodium

Peak Pick

No.	P/V	Wavelength nm.	Abs.	Description
1		281.60	0.411	
2		252.20	0.153	

Figure 3. Maximum wavelength of diclofenac sodium

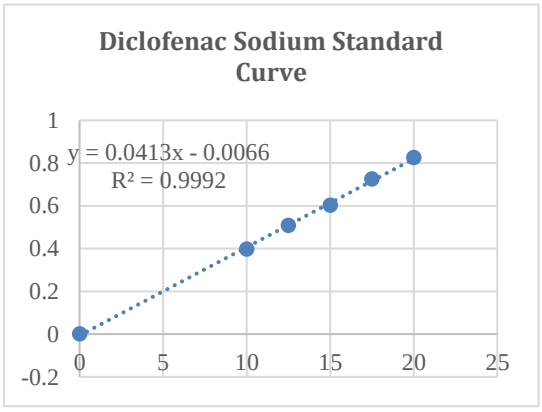

Figure 4. Standard curve of diclofenac sodium

In this study's linear regression value obtained was y=0.0413x-0.0066, with an r-value of 0.9996.

Based on LOD and LOQ calculations in Table 4, the LOD and LOQ value of diclofenac sodium was 1.1625 ppm and 3.8750 ppm.

Precision testing is expressed by % relative standard deviation (%RSD) value. The % RSD value was 0.97% (Table 5). On the accuracy test average % recovery was obtained 95.28%, 92.82%, and 95.48% for the addition of standard solutions of 10 ppm, 15 ppm, and 20 ppm (Table VI).

Determination of content diclofenac sodium was obtained % content for samples A, B, D and G which were 1.46%; 1.82%; 1.03%; and 1.51% (Table VII).

DISCUSSION

In this study, diclofenac sodium standard and 7 samples of jamu pegal linu were analyzed using a stationary phase GF254 silica gel plate and with a mobile phase of a mixture of n-hexane and ethyl acetate in a ratio of 7:3.

According to Firdaus & Utami (2009), qualitative analysis using TLC has several advantages such as short time, ease, and simplicity.

This qualitative test was carried out with 2 replicates to compare the distance of the stain from the standard and the sample formed. The appearance of stain spots was carried out with the help of UV light 254 nm.

Table I. R*f* values of diclofenac sodium standard and 7 samples

No	Code	Result	eluent distance (cm)	*Rf*	Conclusion
		254 nm(cm)		254 nm (cm)	
1	+BP	3.3	4	0.82	Standar
2	A	3.3	4	0.82	+
3	B	3.3	4	0.82	+
4	C	1.5	4	0.37	-
5	D	3.6	4	0.90	+
6	E	-	4	-	-
7	F	-	4	-	-
8	G	3.5	4	0.87	+

Table II. Operating time

Replication	Absorbance (nm)
0	0.392
1	0.392
2	0.392
3	0.393
4	0.393
5	0.393
6	0.393
7	0.394
8	0.394
9	0.394
10	0.394

Table III. Linearity

Concentration (ppm)	Absorbansi
0	0.000
10	0.398
12,5	0.508
15	0.603
17,5	0.725
20	0.826
a	0.0066
b	0.0413
r	0.9996
Linear regression line equation	y = 0.0413x – 0.0066

Table IV. LOD & LOQ

x (ppm)	y	yi	y-yi	$(y-yi)^2$
10	0.398	0.4094	-0.0114	0.0001300
12.5	0.508	0.5127	-0.0047	0.0000216
15	0.603	0.6159	-0.0129	0.0001664
17.5	0.725	0.7192	-0.0059	0.0000342
20	0.802	0.8224	-0.0204	0.0004162
		Σ		0.0007684
		SD		0.0139
		LOD		1.1625
		LOQ		3.8750

R*f* values are calculated using the formula

$$Rf = \frac{distance\ of\ the\ stain\ formed}{eluent\ distance}$$

Based on the results of the calculation, the Rf value of the standard comparison of diclofenac sodium is 0.82. This is different from previous studies which amounted to 0.68 (Setyowati, et al., 2019) and Nasution et al. in 2022 which amounted to 0.85. The difference in Rf values obtained can be caused by several factors such as temperature, plate thickness, plate flatness, and degree of saturation and vapor in the chamber (Primadiamanti, et al., 2018). The difference in Rf values according to Oktaviantari et al. in 2019 can be declared positive if ≤ 0.05. The very large range of differences in Rf values (> 0.05) between standard solutions and samples can be used as a basis that the herbal medicine preparation does not contain diclofenac sodium. However, the absence of diclofenac sodium must be further proven through quantitative analysis (Andini et al., 2022).

The absorbance of 10 ppm standard solution was observed in the wavelength in the range of 200-400 nm., the maximum wavelength of diclofenac sodium obtained was 281 nm.

Operating time was determined at the maximum absorption area by measuring diclofenac sodium standard solution repeatedly at a measurement frequency of once every 1 minute until minute 10 and until a constant absorbance was obtained.

From the results of operating time measurements, a constant absorbance was obtained at minute 0 with an absorbance value of 0.392 nm.

For linearity testing, LOD &LOQ diclofenac sodium standard solutions with concentrations of 10 ppm; 12.5 ppm; 15 ppm; 17.5 ppm, and 20 ppm were measured using UV-Vis spectrophotometry at maximum wavelengths and operating times that had been obtained previously. This study's linear

Table V. Precision

R	Abs	x	$\bar{x}$	$x - \bar{x}$	$(x - \bar{x})^2$
1	0.350	8.63	8.59	0.04	0.0019
2	0.347	8.56		-0.03	0.0008
3	0.353	8.71		0.12	0.0135
4	0.351	8.66		0.07	0.0046
5	0.351	8.66		0.07	0.0046
6	0.351	8.66		0.07	0.0046
7	0.347	8.56		-0.03	0.0008
8	0.344	8.49		-0.10	0.0103
9	0.345	8.51		-0.08	0.0060
10	0.343	8.46		-0.13	0.0159
		Σ			0.0631
		SD			0.0837
		%RSD			0.97 %

Table VI. Accuracy

Sample Code	Standard (ppm)	R	Concentration			% *Recovery*	Average of % *Recovery*
			Cf	*Cu*	*Ca*		
A (500 ppm)	10	1	16.964	7.787	9.700	94.61	95.28
		2	16.988	7.835	9.724	94.12	
		3	17.206	7.787	9.700	97.10	
	15	1	21.855	8.126	14.591	94.09	92.82
		2	21.540	7.448	15.123	93.18	
		3	21.613	7.908	15.027	91.20	
	20	1	26.971	8.005	19.530	97.11	95.48
		2	26.140	8.029	19.361	93.55	
		3	26.528	8.029	19.312	95.79	

Table VII. Diclofenac sodium content in the sample

Sample	Replication	Concentration	Content (mg/1g)	% Content (mg/1g)	Average of % Content
A	0.599	14.663	14.663	1.466	1.46
	0.588	14.397	14.397	1.440	
	0.599	14.663	14.663	1.466	
B	0.756	18.465	18.465	1.846	1.82
	0.744	18.174	18.174	1.817	
	0.737	18.005	18.005	1.800	
D	0.420	10.329	10.329	1.033	1.03
	0.417	10.257	10.257	1.026	
	0.419	10.305	10.305	1.031	
G	0.605	14.809	14.809	1.481	1.51
	0.625	15.293	15.293	1.529	
	0.618	15.123	15.123	1.512	

regression value obtained was y=0.0413x-0.0066, with an r-value of 0.9996. According to Chan et al. (2005), the requirement for a linearity test is ≥ 0.9970, which means that the linearity test results meet the predetermined requirements. Based on the LOD and LOQ calculations in Table 4, the LOD value of diclofenac sodium was 1.1625 ppm. This value is the lowest concentration of diclofenac sodium that can be obtained without an exact quantity. The calculation of the LOQ value obtained

was 3.8750 ppm, which indicates that the lowest concentration of diclofenac sodium obtained precisely was 3.8750 ppm.

Precision testing is expressed by %RSD value. The calculation results show that the standard deviation (SD) value obtained was 0.0837, and the % relative standard deviation (%RSD) value was 0.97%. According to Riyanto (2014), determining a level be can meet the qualifications if the %RSD value is < 2%.

On the accuracy test, the testing process was carried out 3 times replication and obtained an average % recovery of 95.28%, 92.82%, and 95.48% for the addition of standard solutions of 10 ppm, 15 ppm, and 20 ppm. According to Rohman (2014), the accuracy requirement is assessed from the percent recovery, between 80-110%.

Positive samples on qualitative testing using TLC continued with a determination of levels using UV-Vis spectrophotometry. From the calculation results, the calculated values for sample A were 14.663; 14.397; and 14.633, sample B was 18.465; 18.174; and 18.005, sample D was 10.329; 10.257; and 10.305, while sample G was 14.809; 15.293; and 15.123 with the acquisition of the average value of % content for samples A, B, D and G which were 1.46%; 1.82%; 1.03%; and 1.51%.

CONCLUSION

Based on the results of qualitative and quantitative tests using TLC and UV-Vis spectrophotometer, it can be stated that 4 out of 7 samples of jamu pegal linu circulating in the Banjarmasin City area were positive for diclofenac sodium with levels ranging from 10.257 mg/g - 18.465 mg/g (1.026% - 1.846%).

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