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Analysis Of Dexamethasone And Paracetamol Content In Herbal Medicines For Muscle Pain In Ternate City Using Uv-Vis Spectrophotometry

Dessy Trisiani[1], Maghfira Maulani[2], Abulkhair Abdullah[3]*, Muh. Nasir[4]

[1,2,3,4)] Department of Pharmacy, Faculty of Medicine and Health Sciences, Universitas Khairun, Indonesia
e-mail: abulkhairabdullah@unkhair.ac.id

ABSTRACT

Jamu Pegal Linu (herbal medicines for muscle pain) is one of the most commonly consumed traditional herbal drinks to relieve muscle soreness and pain caused by fatigue. The high demand for *jamu* has led to some irresponsible producers adding Active Pharmaceutical Ingredient (API). The APIs commonly found are paracetamol and dexamethasone. This is a violation of the Indonesian Ministry of Health Regulation Number 007 of 2012 concerning Traditional Medicine Registration, which states that traditional medicine must not contain pharmaceutical chemicals. To detect the presence of paracetamol and dexamethasone in *jamu pegal linu* in Ternate City, 10 samples were analyzed qualitatively using Thin Layer Chromatography (TLC) with a mobile phase of chloroform:methanol (9:1), and quantitatively using validated UV-Vis spectrophotometry methods, including linearity, precision, accuracy, LOD, LOQ, and determination of content. Based on TLC testing, 5 samples were suspected to contain paracetamol, and 3 were suspected to contain dexamethasone. The validation results showed that all parameters met the requirements: $R^2 \geq 0.99$, recovery 85.289–92.541%, RSD ≤ 0.79%, LOD 0.204–0.336 µg/mL. Based on qualitative analysis using TLC and quantitative analysis using UV–Visible spectrophotometry, several jamu pegal linu products sold in Ternate City were found to contain active pharmaceutical ingredients. Of the ten samples analyzed, 30% were suspected to contain dexamethasone and 50% were suspected to contain paracetamol. These findings indicate a significant presence of pharmaceutical adulterants in herbal medicines marketed in Ternate City. However, confirmatory analysis using more specific techniques such as High Performance Liquid Chromatography (HPLC) or Liquid Chromatography–Mass Spectrometry (LC-MS) is recommended to strengthen the validity of the results.

Keywords: API, Dexamethasone, Paracetamol, *Jamu Pegal Linu*

Introduction

Traditional medicine has long been an integral part of healthcare culture in Indonesia. One of the most popular types of traditional remedies still widely consumed by the public is *jamu*, a traditional herbal medicine (Ningrum et al., 2023). In North Maluku, 25.2% of the population utilize traditional health services, particularly in the form of ready-made herbal concoctions (Aprilla, 2020). The widespread acceptance of *jamu* among the community stems from its deep-rooted

cultural heritage, passed down from generation to generation, making it readily embraced by society (Adiyasa & Meiyanti, 2021). Among various types of *jamu*, herbal remedies for muscle aches (*jamu pegal linu*) are among the most commonly consumed, used to relieve fatigue-induced soreness and muscle pain (Khoirunnisa et al., 2017). This high demand is primarily due to the nature of many occupations in Indonesia, which involve physical labor and consequently lead to a greater risk of muscle strain (Sukmawati & Sembiring, 2021).

However, this high public interest in *jamu* has led to the emergence of irresponsible manufacturers who illegally add Active Pharmaceutical Ingredient (API) to enhance efficacy (Kamar et al., 2021). According to the Regulation of the Minister of Health of the Republic of Indonesia No. 006/Menkes/Per/I/2012, traditional medicine industries are prohibited from producing traditional medicines that contain synthetic pharmaceutical chemicals with therapeutic effects (Rahmah & Maulida, 2022). The Indonesian Food and Drug Authority (BPOM) has warned the public against consuming herbal medicines containing API due to their potential health risks (Sukmawati & Sembiring, 2021). The most commonly found API in *jamu pegal linu* are dexamethasone and paracetamol (BPOM, 2021).

Uncontrolled and prolonged use of dexamethasone can lead to side effects such as diabetes, osteoporosis, peptic ulcers, and Cushing's syndrome (Chamidah et al., 2021). Similarly, excessive and long-term intake of paracetamol can also pose serious health risks (Chandra et al., 2024). A study by Bamidele et al., (2018), reported that the combination of paracetamol with herbal products has the potential to cause liver damage. Dexamethasone and paracetamol can be analyzed using various methods, one of which is UV-Visible spectrophotometry, known for its selectivity, ability to quantify small amounts of analytes, and accurate data output (Rohmah et al., 2021).

Previous studies conducted in several Indonesian cities, including Cilacap, Jakarta, Kediri, and Semarang, have reported the presence of dexamethasone and paracetamol in jamu pegal linu products (Chamidah et al., 2021; Chandra et al., 2024; Sukmawati & Sembiring, 2021). However, data regarding the adulteration of jamu pegal linu products circulating in Ternate City remain limited, creating a critical research gap considering regional differences in distribution and supervision.

Therefore, this study aims to identify and quantify the presence of dexamethasone and paracetamol in jamu pegal linu products sold in Ternate City using Thin Layer Chromatography (TLC) and validated UV-Visible spectrophotometric methods.

Methodology

Materials and Instruments

The instruments used in this study included a UV lamp (366 nm), an oven (Memmert), an analytical balance, and a UV-Visible spectrophotometer (Thermo Scientific Genesys 150). The materials utilized consisted of dexamethasone reference standard (Dexa Medica), paracetamol reference standard (Dexa Medica),

chloroform (Polylab), methanol (Mercks), dried ginger rhizome (simplicia), and herbal muscle ache remedy samples.

Preparation of 100 ppm Standard Solutions

A total of 50 mg of dexamethasone and paracetamol reference standards were each accurately weighed and dissolved in methanol. The solutions were transferred into separate 50 mL volumetric flasks and shaken until completely dissolved. Methanol was added to reach the volume mark, yielding a stock solution of 1000 ppm. From this, 5 mL of each stock solution was pipetted into separate 50 mL volumetric flasks, diluted to the mark with methanol, and shaken to obtain working solutions with a concentration of 100 ppm.

Determination of Maximum Wavelength (Lambda-Max) of the Standards

Solution (10 ppm) was prepared by diluting 1 mL of the 100 ppm working solution to 10 mL with methanol. The lambda-max of each standard was determined within the wavelength range of 200–400 nm using a UV-Visible spectrophotometer.

Qualitative Analysis Using TLC

Each reference standard (50 mg) was accurately weighed and dissolved in 5 mL of methanol (Khoirunnisa *et al*., 2017). The mobile phase used was a mixture of chloroform:methanol in a 9:1 ratio. The solvent mixture was placed in a TLC chamber, which was then sealed to allow saturation. A TLC plate was activated, and both the reference standard and test solutions were spotted separately onto the plate. The plate was left to dry, then placed in the chamber for development. The mobile phase was allowed to ascend the plate until just below the solvent front limit. The plate was then removed and air-dried. Observations were made under UV light at 366 nm, and the Rf values were calculated (Pradika, 2023). If the standards analyzed were colorless, visualization was enhanced by spraying with 10% H_2SO_4 (Forestryana & Arnida, 2020). A sample was considered positive if it exhibited an Rf value within ±0.05 of the standard Rf value (Wattiheluw & Firdaus, 2023).

Method Validation of UV-Visible Spectrophotometric Analysis

a. Linearity Test

A series of standard solutions of dexamethasone were prepared at concentrations of 2, 3, 4, 5, 6, 7, and 8 μg/mL, while paracetamol standards were prepared at concentrations of 2, 3, 4, 5, 6, 7, 8, and 9 μg/mL. The absorbance of each solution was measured at its respective maximum wavelength (Sukmawati & Sembiring, 2021).

b. Precision Test

The absorbance of 10 ppm standard solutions of dexamethasone and paracetamol was measured six times. The relative standard deviation (RSD) for each was then calculated to assess precision (Minarsih & Roni, 2023).

c. Accuracy Test

The accuracy was evaluated using the standard addition method. A simulated *jamu* solution with a concentration of 100 ppm was prepared in five replicates. The absorbance of the simulated *jamu* was measured first. Then, 10 ppm dexamethasone standard was added to the *jamu* at a ratio of 70:30 (*jamu* to drug), and the absorbance was measured again. The percentage recovery of

dexamethasone was calculated. The same procedure was applied for paracetamol (Arumningtyas, 2022).

d. LOD and LOQ

The LOD and LOQ were calculated using the slope and standard deviation from the calibration curve. The LOD was calculated by dividing three times the standard deviation (x/y) by the slope of the linear regression equation, while the LOQ was calculated using ten times the standard deviation (x/y) divided by the slope (Nursanti Angger Ratnawati et al., 2019).

Quantitative Analysis Using UV-Vis Spectrophotometry

A total of 50 mg of *jamu pegal linu* sample was dissolved in methanol and transferred to a 50 mL volumetric flask, then filled to the mark with methanol. From this solution, 0.1 mL was pipetted and diluted to 10 mL with methanol. The absorbance of the test solution was measured using a UV-Vis spectrophotometer at the maximum wavelengths of the dexamethasone and paracetamol reference standards. The analysis was conducted in triplicate. The resulting absorbance values were processed to determine the concentrations of dexamethasone and paracetamol in the sample (Ryansyah, 2022).

Result and Discussion

1. Identification of *Jamu Pegal Linu* Samples

Initial identification of ten *jamu pegal linu* samples was conducted through visual inspection of product packaging and verification of registration numbers using the official website of the Indonesian Food and Drug Authority (BPOM RI). The inspection results indicated that none of the samples displayed batch numbers, and the registration numbers stated on the packaging were not found in the BPOM RI database. In addition, only five out of ten samples included expiration dates.

These findings indicate that the *jamu pegal linu* samples examined in this study did not comply with the administrative and regulatory requirements stipulated in the Regulation of the Minister of Health of the Republic of Indonesia Number 006/Menkes/Per/I/2012. Such non-compliance reflects inadequate adherence of producers to existing regulations and may increase potential risks to consumer safety.

2. Qualitative Analysis Using TLC

This qualitative analysis aimed to detect the presence of dexamethasone and paracetamol, which are classified as unapproved pharmaceutical substances in *jamu pegal linu* products. Before use, the TLC plates were activated to eliminate moisture adsorbed on the surface. The mobile phase used in this analysis was a mixture of chloroform:methanol in a 9:1 ratio. The selection of solvents was based on the solubility properties listed in the Indonesian Pharmacopoeia and references from relevant scientific literature.

Table 1. Detection Results of API in *Jamu Pegal Linu* Using TLC

Sample	Dexamethasone	Paracetamol
Jamu 1	+	+
Jamu 2	+	+
Jamu 3	-	-
Jamu 4	-	+
Jamu 5	-	+
Jamu 6	-	-
Jamu 7	+	-
Jamu 8	-	-
Jamu 9	-	+
Jamu 10	-	-

TLC analysis under 366 nm UV light suggested several samples contained dexamethasone and paracetamol. The dexamethasone standard (Rf 0.64) closely matched samples *Jamu* 1 (0.61), *Jamu* 2 (0.61), and *Jamu* 7 (0.64), all appearing as dark purple spots. Similarly, the paracetamol reference (Rf 0.76) was comparable to five samples: *Jamu* 1 (0.78), *Jamu* 2 (0.78), *Jamu* 4 (0.72), *Jamu* 5 (0.76), and *Jamu* 9 (0.75). The close proximity of these sample Rf values (within $\pm$0.05 of the standards) supported the identification.

For initially colorless spots, the TLC plates were sprayed with 10% sulfuric acid (H_2SO_4). The use of sulfuric acid was due to its function as a reducing agent that disrupts chromophore groups, shifting their absorbance to longer wavelengths and thereby enhancing visibility. It also intensifies fluorescence, allowing the spots to appear brighter and more distinct when observed (Adysti, 2022).

The results demonstrated that five samples exhibited Rf values consistent with the paracetamol standard, while three samples showed Rf values corresponding to the dexamethasone standard. These findings indicate the presence of paracetamol and dexamethasone in several *jamu pegal linu* samples analyzed in this study.

These findings are consistent with previous studies reporting the presence of pharmaceutical adulterants, particularly paracetamol and dexamethasone, in *jamu pegal linu* products distributed in various regions of Indonesia. Several studies have documented that the addition of active pharmaceutical ingredients (APIs) to traditional herbal medicines remains prevalent, especially in products lacking official marketing authorization. The consistency between the present findings and previous reports suggests that the adulteration of herbal medicines with APIs is not an isolated occurrence but continues to represent a widespread public health concern.

3. Quantitative Analysis Using UV-Visible Spectrophotometry

a. Determination of Lambda-Max

The lambda-max was determined using a 10 ppm concentration of each standard solution. The resulting spectra showed that the lambda-max of

dexamethasone occurred at 239 nm, while that of paracetamol was observed at 248 nm (see Figure 1). These wavelengths were subsequently used for method validation and analysis of *jamu pegal linu* samples. Theoretically, the maximum absorption of dexamethasone is expected at 239 nm, and paracetamol at 244 nm (Dirjen POM, 2014). Minor wavelength shifts may be attributed to differences in solvent composition and instrumental conditions during measurement.

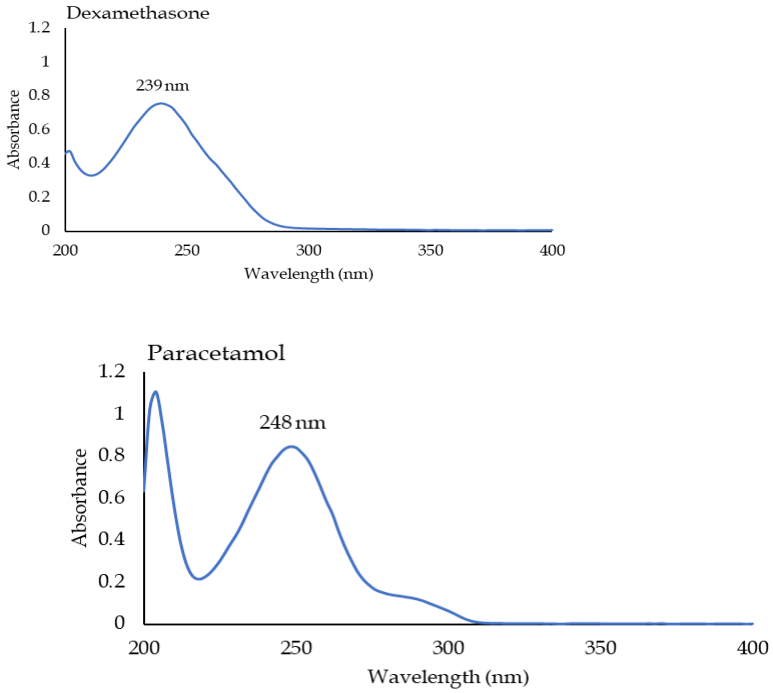

Figure 1. Absorption Spectrum of Lambda-Max for Dexamethasone and Paracetamol

b. Linearity Test

Linearity refers to a method's ability to produce results that are directly proportional to the concentration of analytes in a sample. Linearity is typically assessed by the correlation coefficient (r) of the linear regression curve $y = bx + a$, with ideal values of $a = 0$ and $r = +1$ or -1. In this study, linearity was evaluated as part of method validation to determine the capability of the spectrophotometric method in detecting the concentrations of paracetamol and dexamethasone in the samples. The calibration curves demonstrated strong linearity, with R^2 values ≥ 0.99 (see Figure 2). These results confirm that the UV-Visible spectrophotometric method is suitable for analyzing API in *jamu pegal linu* products.

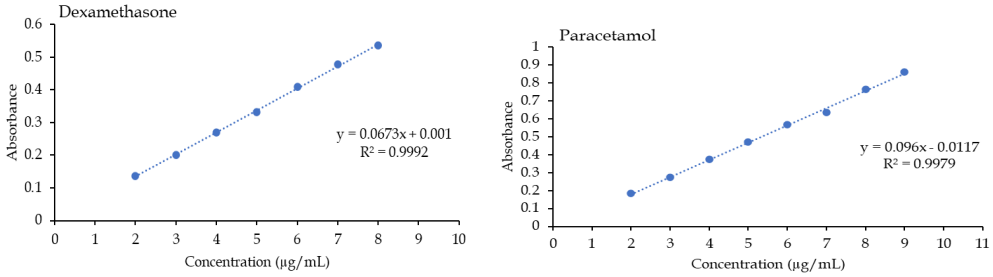

Figure 2. Calibration Curves of Dexamethasone and Paracetamol

c. Precision Test

Precision is the degree of agreement among repeated measurements under the same conditions (Khairunnisa et al., 2022). The precision test was conducted according to ICH guidelines using one concentration level (10 ppm) with six replicates (Zaenudin, 2022). As shown in Table 2, the precision test yielded an RSD of 0.59% for paracetamol and 0.79% for dexamethasone, both within the acceptable limit of ≤2%. These results indicate that the UV-Vis spectrophotometric method demonstrates good precision and is reliable for further analysis.

Table 2. Method Validation Results for Precision Test

Standard	Initial Conc. (ppm)	Absorbance	Final Conc. (ppm)	Mean (ppm)	SD	RSD (%)
Dexamethasone	10	0,694	10,295	10,349	0,082	0,79
		0,705	10,459			
		0,704	10,444			
		0,692	10,265			
		0,697	10,295			
		0,694	10,340			
Paracetamol	10	0,985	10,382	10,387	0,062	0,59
		0,989	10,423			
		0,981	10,340			
		0,978	10,309			
		0,995	10,486			
		0,985	10,382			

d. Accuracy Test

Accuracy refers to the degree of closeness between the measured value and the true value. As shown in Table 3, the average recovery rate was 85.289% for dexamethasone and 92.541% for paracetamol. These values fall within the acceptable accuracy range of 80–110% at a concentration of 10 ppm. Accuracy testing was performed using a simulation method, in which simulated *jamu* was made with ginger and measured for absorbance. The recovery rate was calculated using a predetermined regression equation. The ratio of *jamu* to added standard in

the simulated sample was 70:30. Ginger was selected for simulation as it closely represents the major composition of the *jamu pegal linu* samples analyzed. Based on these findings, the UV-Vis spectrophotometric method used in this study can be considered accurate and reliable for quantitative analysis.

Table 3. Method Validation Results for Accuracy Test

Standard	Abs. of Jamu (100 ppm)	Abs. of Paracetamol Standard (10 ppm)	Jamu Conc. (ppm)	Jamu + Std Conc. (ppm)	% Recovery	Mean Recovery (%)
Dexamethasone	0,142	0,735	20,936	10,904	88,122	85,289
	0,138	0,708	20,341	10,503	84,695	
	0,148	0,731	21,827	10,845	86,627	
	0,146	0,706	21,530	10,473	83,209	
	0,140	0,704	20,638	10,444	83,803	
Paracetamol	0,089	0,990	1,048	10,434	93,854	92,541
	0,097	0,998	1,132	10,517	93,854	
	0,093	0,981	1,090	10,340	92,5	
	0,094	0,983	1,101	10,361	92,604	
	0,094	0,957	1,101	10,090	89,895	

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

As shown in Table 4, the lowest detectable concentration (LOD) was 0.2049 µg/mL for dexamethasone and 0.3365 µg/mL for paracetamol. The limit of quantification (LOQ), representing the lowest concentration that can be reliably quantified, was determined to be 0.6211 µg/mL for dexamethasone and 1.1218 µg/mL for paracetamol.

Table 4. Results of LOD and LOQ Analysis

Standard	SD	LOD (µg/mL)	LOQ (µg/mL)
Dexamethasone	0,0041	0,2049	0,6211
Paracetamol	0,0107	0,3365	1,1218

f. Determination of the Concentration of API

Quantitative analysis through content determination represents the final stage in analyzing the presence of API in traditional herbal pain relief products circulating in Ternate City. The method employed in this analysis was UV-Visible spectrophotometry, using the absorbance value of the analyte as the analytical basis. A compound can be analyzed by spectrophotometry if it is an organic substance that possesses functional groups capable of absorbing light, known as chromophores and auxochromes. Paracetamol contains both chromophore and auxochrome groups its chromophore is a benzene ring, while the auxochrome groups consist of hydroxyl and amide groups. Dexamethasone, on the other hand, features a cyclohexanone

chromophore and hydroxyl auxochromes. These functional groups enable both compounds to be analyzed using UV-Visible spectrophotometry.

Table 5. Determination of API

Sample	Absorbance		Concentration (ppm)		Content (mg)	
	239 nm	248 nm	239 nm	248 nm	239 nm	248 nm
Jamu 1	0,021	0,022	0,300	0,351	1,503	1,755
Jamu 2	0,035	0,027	0,508	0,403	2,543	2,015
Jamu 3	-	-	-	-	-	-
Jamu 4	-	0,712	-	7,538	-	37,692
Jamu 5	-	0,013	-	0,257	-	1,286
Jamu 6	-	-	-	-	-	-
Jamu 7	0,029	-	0,424	-	2,122	-
Jamu 8	-	-	-	-	-	-
Jamu 9	-	0,009	-	0,215	-	1,078
Jamu 10	-	-	-	-	-	-

The results of the analysis of paracetamol and dexamethasone in *jamu pegal linu* products sold in Ternate City indicate that 8 out of 10 samples contained measurable concentrations. Moreover, because sample *Jamu* 3 was found to contain five whole tablets one of which was suspected to be a paracetamol tablet reanalysis was conducted on the individual tablet without mixing it with the others. The subsequent test showed that the single tablet had a higher concentration (41.026 mg) compared to the previously mixed sample, which contained only 28.630 mg. Based on this finding, it can be concluded that *Jamu* 3 is strongly suspected of containing paracetamol. Measurement results further identified samples suspected of containing paracetamol namely, *Jamu* 1, 2, 4, 5, and 9 with respective concentrations of 1.755 mg, 2.015 mg, 37.692 mg, 1.286 mg, and 1.078 mg. Meanwhile, samples suspected of containing dexamethasone were *Jamu* 1, 2, and 7, with concentrations of 1.503 mg, 2.543 mg, and 2.122 mg, respectively.

The results of API concentration determination revealed that several *jamu pegal linu* samples contained paracetamol and dexamethasone at varying levels. One sample was found to contain paracetamol at a concentration of 37.692 mg, which approaches the single-dose level of conventional pharmaceutical preparations. This finding strongly suggests the intentional addition of APIs to enhance therapeutic effects rapidly.

The findings from this analysis of traditional herbal medicines in Ternate City suggest that a number of traditional medicine producers continue to violate the regulations stated RI Minister of Health Regulation No.006/Menkes/Per/I/2012 on the Traditional Medicine Industry and Enterprises. Article 37 clearly prohibits the manufacture of traditional medicines that contain synthetic chemical substances or isolated active compounds with medicinal properties.

Conclusion

Based on qualitative analysis using TLC and quantitative analysis using UV–Visible spectrophotometry, several jamu pegal linu products circulating in Ternate City were confirmed to contain Active Pharmaceutical Ingredients (APIs), namely dexamethasone and paracetamol. Of the ten samples analyzed, three samples (30%) were confirmed to contain dexamethasone, while five samples (50%) were confirmed to contain paracetamol.

Declaration of Competing Interest

The authors declare that this research on the presence of medicinal chemical substances in traditional "jamu pegal linu" products was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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