

## Analysis Study of Methampyrone in Jamu Pegal Linu Circulating in Semarang City using Fourier Transform Infrared (FTIR) Spectrophotometry and UV-Vis Spectrophotometry

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**Abstract: Background:** Traditional medicine (jamu) has been widely known as an alternative medicine because its side effects are mild, easy to obtain, and cheaper than synthetic medicines. One of the herbal medicines used as a pain reliever in Indonesia is called Jamu Pegal linu. Analgesic drugs are often added illegally to herbal remedies to treat aches and pains. Methampyrone is an example of a medicinal chemical that can most likely be added to jamu pegal linu. The National Food and Drug Administration reported the discovery of contamination in the form of medicinal chemicals. The distribution of herbal medicines containing medicinal chemicals that are dangerous to the public is prohibited based on Minister of Health Regulation no. 007 of 2012. **Aim:** This study aims to determine the content and levels of the medicinal chemical methampyrone in jamu pegal linu in Semarang City. In this study, jamu pegal linu sold in Semarang City will be analyzed for the medicinal chemical content of methampyrone. **Material and Methods:** This research is an experimental study to qualitatively and quantitatively analyze the medicinal chemicals of the drug methampyrone in jamu pegal linu. The qualitative analysis method uses FTIR to identify functional groups. The quantitative analysis method uses UV-Vis Spectrophotometry which has been validated to determine the levels of methampyrone contained in jamu pegal linu. **Results:** The results of qualitative analysis tests using FTIR show that methampyrone contains functional groups such as C=O (amide), C=C (aromatic), C-H, O=S=O, C-O, S=O, and C-H out-of-plane. The readings from the fifth samples (A, B, N, I, and L) were positive for methampyrone due to the presence of these functional groups, which are characteristic of methampyrone. Validation of the analytical method using UV-Vis Spectrophotometry demonstrates its suitability for quantifying methampyrone levels in jamu pegal linu. The method validation parameters include a correlation coefficient ( $R_2$ ) of 0.9997; limit of detection (LOD) of 0.275966 mg/L; limit of quantification (LOQ) of 0.9198867 mg/L; precision expressed as % RSD of 0.1444%; and accuracy indicated by %recovery of 82,7544%; 84,005%; and 85,721%. The results from the validated analysis method reveal the presence of methampyrone in jamu pegal linu across the five samples (A, B, N, I, and L), with concentrations in each sample measured at  $3,594 \pm 0,002\%$ ;  $1,507 \pm 0,003\%$ ;  $1,386 \pm 0,003\%$ ;  $2,900 \pm 0,003\%$ ; and  $31,870 \pm 0,028\%$ .

**Keywords:** Methampyrone, Jamu Pegal Linu, Fourier Transform Infrared Spectrophotometry (FTIR), UV-Vis Spectrophotometry

## INTRODUCTION

Jamu or traditional Indonesian medicine is one of Indonesia's cultural heritages which has been consumed by almost all Indonesian people, especially in rural areas, for generations. In recent years, cases of mixing herbal medicines with medicinal chemicals have increased. In general, herbal medicine cannot cure or have an effect on the body instantly, if this happens, it is very likely that the herbal medicine contains medicinal ingredients.

In 2021, BPOM carried out sampling and testing of traditional medicinal products and they were found to contain BKO. One of the traditional medicinal products that BKO adds the most is herbal medicine for rheumatic pain (BPOM, 2022). BPOM (2017) found 39 traditional medicine products containing BKO and 28 products did not have BPOM distribution permits. According to BPOM findings (2006), the BKO that often contaminates traditional medicines for rheumatic pain are NSAID class drugs, such as phenylbutazone, methampyrone, diclofenac sodium, piroxicam, paracetamol, or dexamethasone.

Jamu pegal linu is a herbal medicine that is used to reduce pain, fatigue, muscle and bone pain, improve blood circulation and reduce pain throughout the body (Fatimah et al., 2017). Methampyrone is a chemical that is often used as an analgesic and antipyretic drug. However, by rogue herbal medicine manufacturers, these chemicals are added to herbal products to treat aches and pains. Basically methampyrone is not a dangerous chemical drug, however, long-term and continuous use of methampyrone and unknown levels can cause quite serious side effects 4 (Fatimah et al., 2017).

Based on the description above regarding the many cases of adding medicinal chemicals to herbal medicine, researchers are interested in conducting methampyrone analysis research using the FTIR method and UV-Vis spectrophotometry on herbal medicines for stiffness in Semarang City. In this research, qualitative and quantitative analysis will be carried out. Qualitative analysis uses the FTIR method. The FTIR method is an initial identification to determine whether or not the chemical drug methampyrone is present by looking at the structure or functional

groups of standards and samples. Quantitative analysis was carried out using UV-Vis spectrophotometry to calculate the levels of medicinal chemicals in the samples.

## MATERIAL and METHODS

This research is an experimental study to qualitatively and quantitatively analyze the medicinal chemicals of the drug methampyrone in jamu pegal linu. The qualitative analysis method uses FTIR to identify functional groups. The quantitative analysis method uses UV-Vis Spectrophotometry which has been validated to determine the levels of methampyrone contained in jamu pegal linu.

### Time and Location

Research using UV-Vis spectrophotometry and FTIR spectroscopy (Fourier Transform Infrared Spectroscopy) methods was carried out at the STIFAR Instrument Laboratory "Yayasan Pharmasi Semarang".

### Equipment and Materials

The equipment used in this study were micropipette, 10 mL measuring pipette, dropper pipette, bulb, Erlenmeyer, measuring flask (10 mL, 50 mL and 100 mL), cuvette, beaker glass, stir bar, metal spatula, horn spoon, funnel, filter paper (Whatman No. 1), watch glass, analytical scales, vials, double beam UV-Vis spectrophotometry equipment, and FTIR.

The materials used for this study were 5 samples of herbal medicine (jamu pegal linu), comparison standard methampyrone (BPOM), HCl 0.1 N.

### Qualitative Analysis by FTIR Spectrophotometry

The methampyrone standard powder and herbal sample powder were each placed on a sample container (ATR crystal) and pressed using a micrometer-controlled compression clamp to ensure proper contact and observed at a wavelength of 4000-650  $\text{cm}^{-1}$ . The ATR crystal was cleaned with 96% ethanol to remove any sample residue.

## Method Validation

### Linearity

The linearity test is based on the coefficient of determination ( $R^2$ ) in the linear regression equation of the methampyrone calibration curve made from 5 concentration variations, namely 8, 11, 14, 17, and 20 ppm.

### LOD and LOQ

The LoD and LoQ tests were determined by statistically calculating the linear regression equation of the methampyrone calibration curve obtained from the linearity test results.

### Precision

The precision of the method is checked and verified by its repeatability. Repetition is measure each sample 6 times on the same day.

### Accuracy

Accuracy testing was carried out by adding methampyrone standard solution with concentrations of 8, 14, and 20 ppm to herbal medicine samples in a 1:1 ratio in a cuvette. The accuracy of the method was assessed based on the percentage recovery.

## Quantitative Analysis by UV-Vis Spectrophotometry

### Preparation of Standard Solution

Standard of methampyrone was weighed 10 mg and dissolved in absolute HCl 0.1 N up to 10 mL to obtain a standard solution with a concentration of 1000 ppm. Each standard solution was pipetted as much as 1 mL, and the volume was sufficient to obtain a final concentration of 100 ppm. The standard solution is then stored in a closed glass container for further tests.

### Preparation of Methampyrone Standard Curve Solution

100 ppm methampyrone stock solution was taken with a volume of 0.8 mL; 1.1 mL; 1.4 mL; 1.7 mL; and 2 mL and put into a 10 mL volumetric flask. Next, the volume of each solution was made up to 10 mL with HCl 0.1 N to obtain a series of concentrations of the standard solution of paracetamol 8, 11, 14, 17, dan 20 ppm. Absorption of each

concentration of the standard solution was measured using a UV-visible spectrophotometer at a maximum wavelength.

### Sample Solution Preparation

The sample was weighed as much as 50 mg, put into an Erlenmeyer flask and 0.1 N HCl added, then shaken until homogeneous. After shaking, put it in a 50 mL measuring flask by filtering it using filter paper and adding 0.1 N HCl solvent until the mark is indicated. 5 mL of the sample solution was pipetted, put into a 10 mL measuring flask and 0.1 N HCl was added to the mark.

### Determination of Maximum Wavelength

The absorbance of the sample solution was measured for 6 repetitions at the maximum wavelength and using 0.1 N HCl blank. The level of methampyrone in the sample was calculated with linear regression equation  $y = bx \pm a$ .

### Data Analysis

Qualitative analysis is carried out by observing the shape of the spectrum to identify specific peaks that indicate the type of functional group in the compound. The spectrum of herbal medicine samples is compared with the spectrum of reference standards.

The level of the herbal sample is known based on the standard curve equation  $y = bx \pm a$ , where y is the absorbance value and x is the measured level. From the sample reading, it was found that the absorbance as y and x was the measured level with the level of w / v (mg/L)

## RESULT AND DISCUSSION

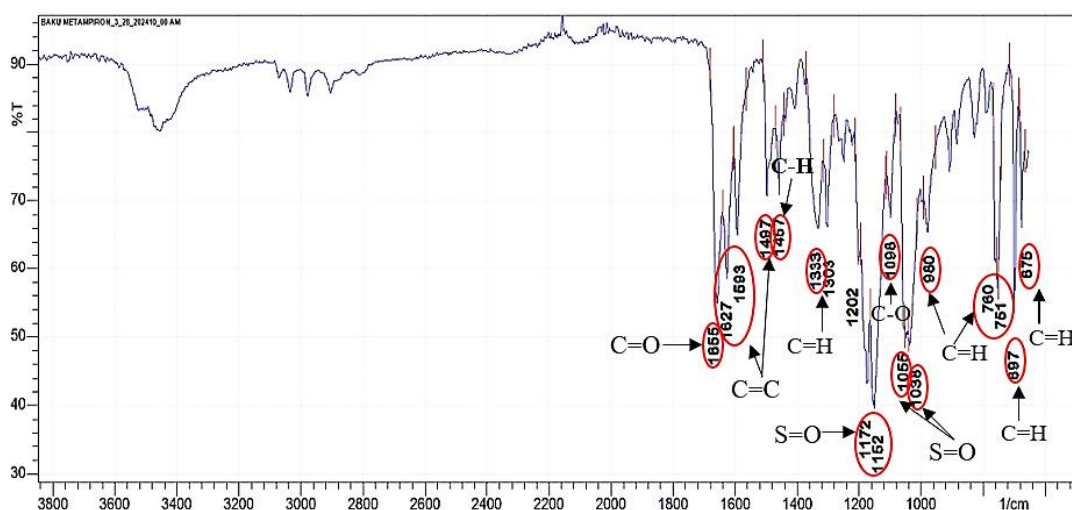


Figure 1. FTIR test results on methampyrone standard

Table 1. Results of FTIR Functional Group Identification on the Methampyrone Standard

| Standard Wavenumbers of Methampyrone (cm <sup>-1</sup> ) | Wavenumber Range (cm <sup>-1</sup> ) | Interpretation of Functional Groups |
|--|--------------------------------------|-------------------------------------|
| 1655   | 1680-1630                            | C=O (amide)                         |
| 1627   | 1680-1600                            | C=C (alkene)                        |
| 1593   | 1615-1580                            | C=C (aromatic)                      |
| 1497   | 1510-1450                            | C=C (aromatic)                      |
| 1457   | 1470-1430                            | C-H                                 |
| 1333   | 1350-1330                            | C-H                                 |
| 1172   | 1200-1100                            | O=S=O                               |
| 1152   | 1200-1100                            | O=S=O                               |
| 1098   | 1140-1070                            | C-O                                 |
| 1055   | 1070-1030                            | S=O                                 |
| 1038   | 1070-1030                            | S=O                                 |
| 980  | 1000-650                             | C-H out-of-plane (alkene)           |
| 760  | 1000-650                             | C-H out-of-plane (alkene)           |
| 751  | 1000-650                             | C-H out-of-plane (alkene)           |
| 697  | 1000-650                             | C-H out-of-plane (alkene)           |
| 675  | 1000-650                             | C-H out-of-plane (alkene)           |

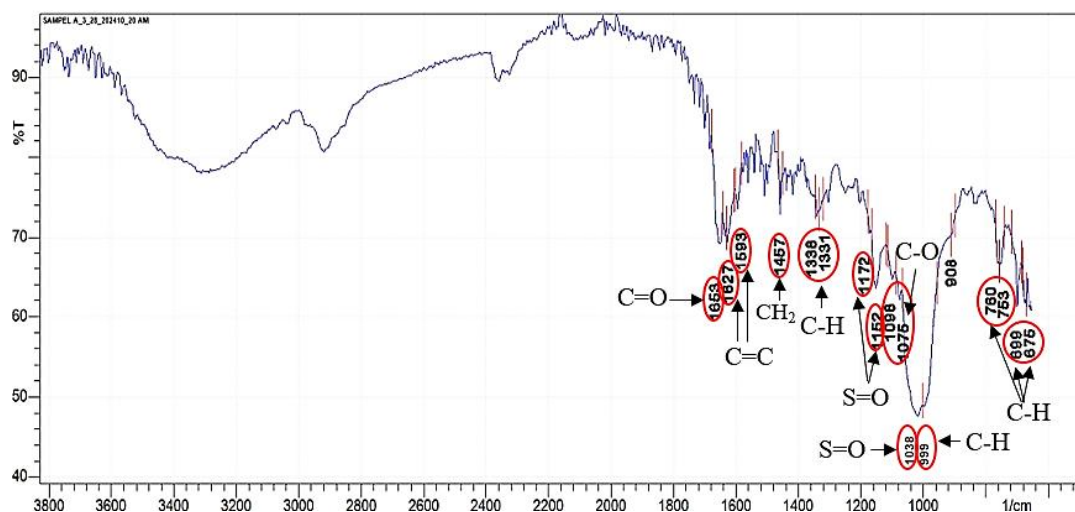


Figure 2. FTIR Test Results for Sample A

Table 2. Results of Functional Group Identification for Sample A

| Wavenumber of Sample A ( $\text{cm}^{-1}$ ) | Wavenumber Range ( $\text{cm}^{-1}$ ) | Interpretation of Functional Groups |
|---|---------------------------------------|-------------------------------------|
| 1653  | 1680-1630                             | C=O (amide)                         |
| 1627  | 1680-1600                             | C=C (alkene)                        |
| 1593  | 1615-1580                             | C=C (aromatic)                      |
| 1457  | 1470-1430                             | C-H                                 |
| 1338, 1331                                  | 1350-1330                             | C-H                                 |
| 1172  | 1200-1100                             | O=S=O                               |
| 1152  | 1200-1100                             | O=S=O                               |
| 1098, 1076                                  | 1140-1070                             | C-O                                 |
| 1038  | 1070-1030                             | S=O                                 |
| 999   | 1000-650                              | C-H out-of-plane (alkene)           |
| 760   | 1000-650                              | C-H out-of-plane (alkene)           |
| 753   | 1000-650                              | C-H out-of-plane (alkene)           |
| 699   | 1000-650                              | C-H out-of-plane (alkene)           |
| 675   | 1000-650                              | C-H out-of-plane (alkene)           |

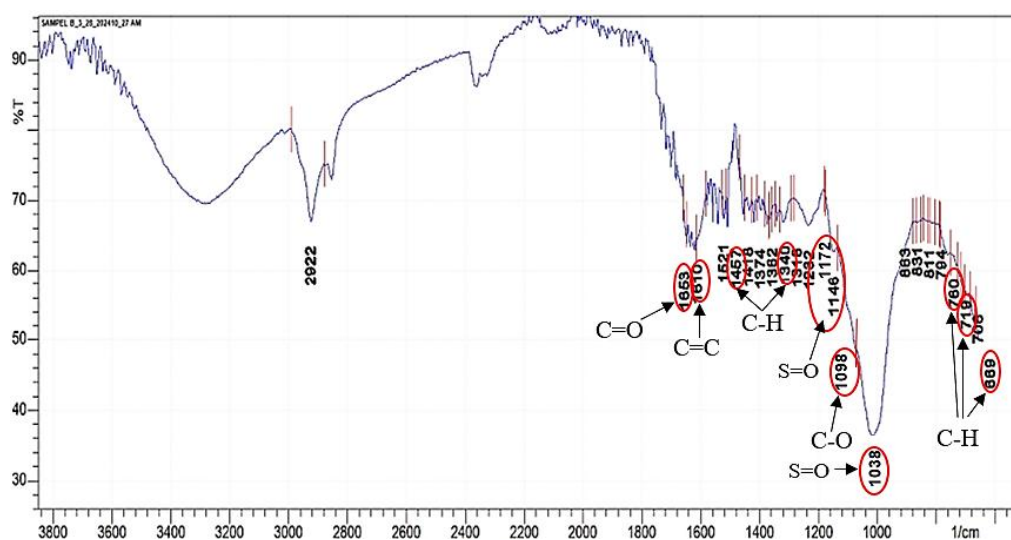


Figure 3. FTIR Test Results for Sample B

Table 3. Results of Functional Group Identification for FTIR Test Sample B

| Wavenumber of Sample B (cm <sup>-1</sup> ) | Wavenumber Range (cm <sup>-1</sup> ) | Interpretation of Functional Groups |
|--|--------------------------------------|-------------------------------------|
| 1653                                       | 1680-1630                            | C=O (amide)                         |
| 1610                                       | 1680-1600                            | C=C (alkene)                        |
| 1457                                       | 1470-1430                            | C-H                                 |
| 1340                                       | 1350-1330                            | C-H                                 |
| 1172                                       | 1200-1100                            | O=S=O                               |
| 1146                                       | 1200-1100                            | O=S=O                               |
| 1098                                       | 1140-1070                            | C-O                                 |
| 1038                                       | 1070-1030                            | S=O                                 |
| 760  | 1000-650                             | C-H out-of-plane (alkene)           |
| 719  | 1000-650                             | C-H out-of-plane (alkene)           |
| 669  | 1000-650                             | C-H out-of-plane (alkene)           |

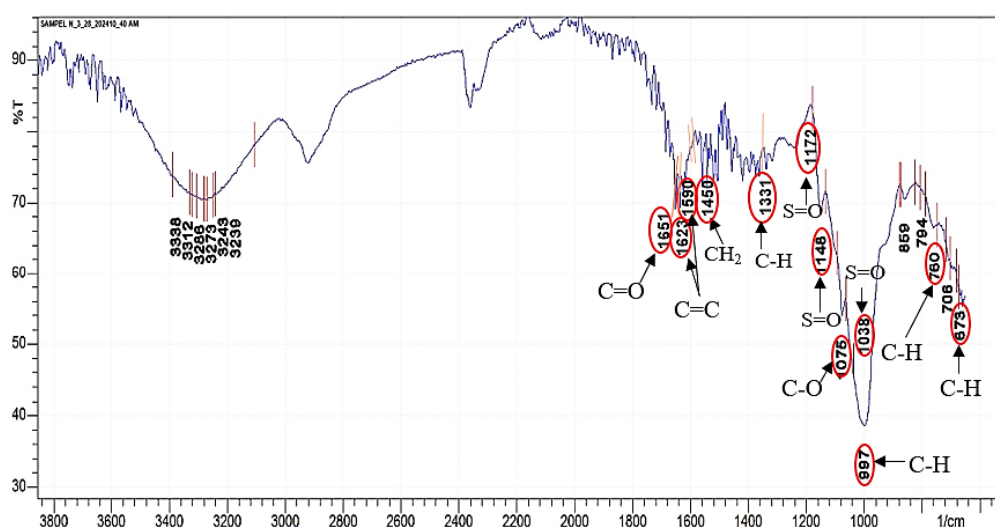


Figure 4. FTIR Test Results for Sample N

Table 4. Results of FTIR Functional Group Identification for Sample N

| Wavenumber of Sample N (cm <sup>-1</sup> ) | Wavenumber Range (cm <sup>-1</sup> ) | Interpretation of Functional Groups |
|--|--------------------------------------|-------------------------------------|
| 1651                                       | 1680-1630                            | C=O (amide)                         |
| 1623                                       | 1680-1600                            | C=C (alkene)                        |
| 1590                                       | 1615-1580                            | C=C (aromatic)                      |
| 1450                                       | 1470-1430                            | C-H                                 |
| 1333                                       | 1350-1330                            | C-H                                 |
| 1148                                       | 1200-1100                            | O=S=O                               |
| 1172                                       | 1200-1100                            | O=S=O                               |
| 1075                                       | 1140-1070                            | C-O                                 |
| 1038                                       | 1070-1030                            | S=O                                 |
| 997  | 1000-650                             | C-H out-of-plane (alkene)           |
| 760  | 1000-650                             | C-H out-of-plane (alkene)           |
| 673  | 1000-650                             | C-H out-of-plane (alkene)           |

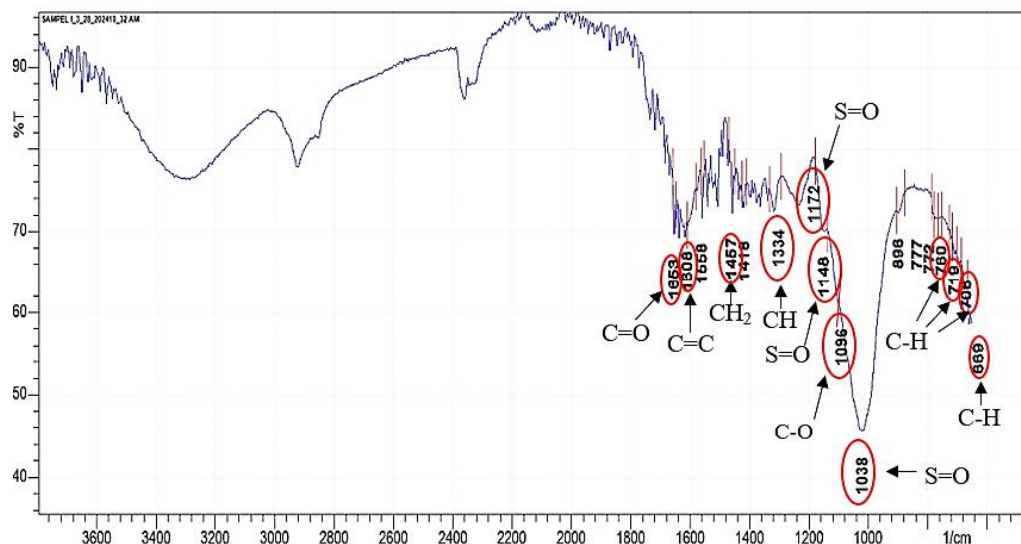


Figure 5. FTIR Test Results for Sample I

Table 5. Results of FTIR Function Group Identification for Sample I

| Wavenumber of Sample I (cm <sup>-1</sup> ) | Wavenumber Range (cm <sup>-1</sup> ) | Interpretation of Functional Groups |
|--|--------------------------------------|-------------------------------------|
| 1653                                       | 1680-1630                            | C=O (amide)                         |
| 1608                                       | 1680-1600                            | C=C (alkene)                        |
| 1457                                       | 1470-1430                            | C-H                                 |
| 1334                                       | 1350-1330                            | C-H                                 |
| 1172                                       | 1200-1100                            | O=S=O                               |
| 1148                                       | 1200-1100                            | O=S=O                               |
| 1096                                       | 1140-1070                            | C-O                                 |
| 1038                                       | 1070-1030                            | S=O                                 |
| 760  | 1000-650                             | C-H out-of-plane (alkene)           |
| 719  | 1000-650                             | C-H out-of-plane (alkene)           |
| 706  | 1000-650                             | C-H out-of-plane (alkene)           |
| 669  | 1000-650                             | C-H out-of-plane (alkene)           |

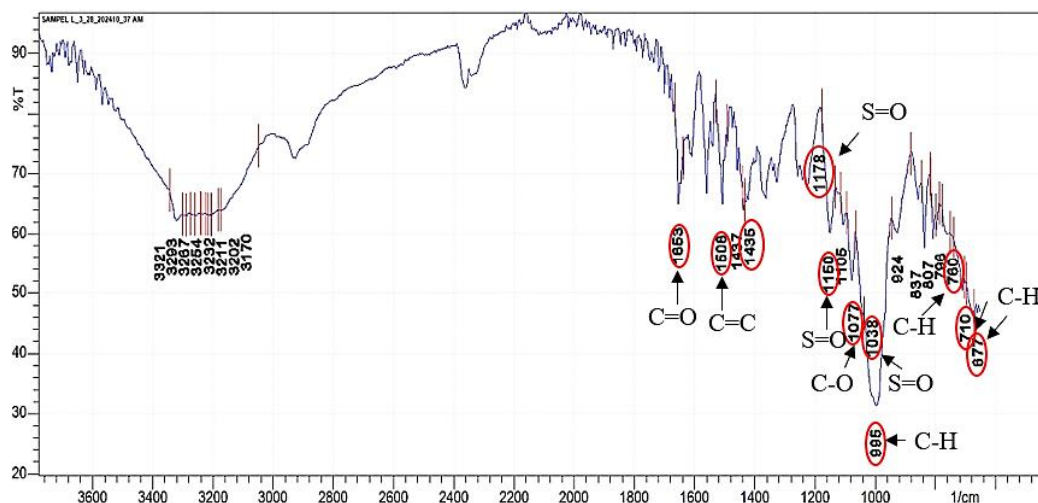


Figure 6. FTIR Test Results for Sample L



Table 6. Results of FTIR Function Group Identification for Sample L

| Wavenumber of Sample L (cm <sup>-1</sup> ) | Wavenumber Range (cm <sup>-1</sup> ) | Interpretation of Functional Groups |
|--|--------------------------------------|-------------------------------------|
| 1653                                       | 1680-1630                            | C=O (amide)                         |
| 1508                                       | 1510-1450                            | C=C (aromatic)                      |
| 1435                                       | 1470-1430                            | C-H                                 |
| 1178                                       | 1350-1140                            | O=S=O                               |
| 1150                                       | 1350-1140                            | O=S=O                               |
| 1077                                       | 1140-1070                            | C-O                                 |
| 1038                                       | 1070-1030                            | S=O                                 |
| 995  | 1000-650                             | C-H out-of-plane (alkene)           |
| 760  | 1000-650                             | C-H out-of-plane (alkene)           |
| 710  | 1000-650                             | C-H out-of-plane (alkene)           |
| 677  | 1000-650                             | C-H out-of-plane (alkene)           |

## Quantitative Analysis Using UV-Vis Spectrophotometry

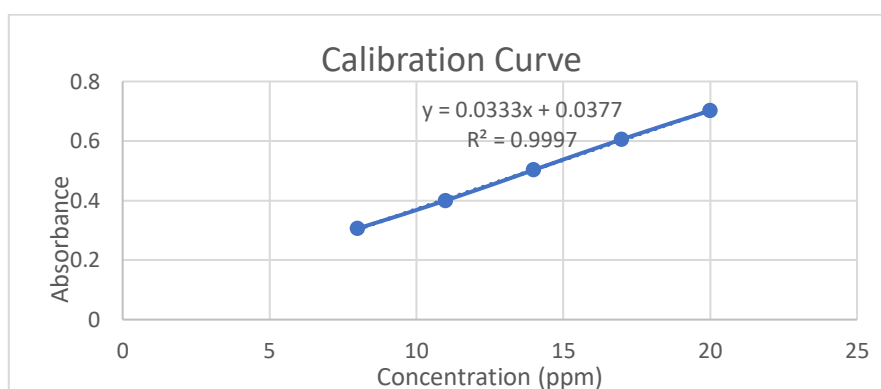


Figure 7. Methampyrone Calibration Curve

Table 7. Results of LOD and LOQ tests

| Concentration (x)    | Absorbance (y) | y'     | y-y'    | (y-y') <sup>2</sup> |
|----------------------|----------------|--------|---------|---------------------|
| 8                    | 0,306          | 0,3041 | 0,0019  | 0,00000361          |
| 11                   | 0,400          | 0,404  | -0,004  | 0,00001600          |
| 14                   | 0,503          | 0,5039 | -0,0009 | 0,00000081          |
| 17                   | 0,606          | 0,6038 | 0,0022  | 0,00000484          |
| 20                   | 0,702          | 0,7037 | -0,0017 | 0,00000289          |
| Total number         |                |        |         | 0,00002815          |
| Sy/x                 |                |        |         | 0,0030632           |
| Limit detection      |                |        |         | 0,275966            |
| Limit quantification |                |        |         | 0,9198867           |

Table 8. Precision test results

| Sample  | Concentration (ppm) | SD     | RSD (%) |
|---------|---------------------|--------|---------|
| A       | 17,9720             | 0,0123 | 0,0682  |
| B       | 7,5365              | 0,0155 | 0,2058  |
| N       | 6,9309              | 0,0164 | 0,2373  |
| I       | 14,4985             | 0,0164 | 0,1134  |
| L       | 15,9349             | 0,0155 | 0,0973  |
| Average |                     | 0,0152 | 0,1444  |

Table 9. Accuracy test results

| Sample  | Measured Concentration (mg/L) |           |           | Theoretical concentration (mg/L) | %Recovery |           |           |
|---------|-------------------------------|-----------|-----------|----------------------------------|-----------|-----------|-----------|
|         | 8 (mg/L)                      | 14 (mg/L) | 20 (mg/L) |                                  | 8 (mg/L)  | 14 (mg/L) | 20 (mg/L) |
| A       | 24,413                        | 29,458    | 34,584    | 17,977                           | 80,455    | 82,011    | 82,933    |
| B       | 14,493                        | 20,069    | 25,224    | 7,527                            | 87,087    | 89,590    | 88,488    |
| N       | 13,351                        | 18,382    | 23,492    | 6,926                            | 80,314    | 81,829    | 82,258    |
| I       | 20,960                        | 25,855    | 32,221    | 14,514                           | 80,581    | 81,010    | 88,539    |
| L       | 22,842                        | 29,068    | 33,863    | 15,945                           | 85,335    | 85,586    | 86,386    |
| Average |                               |           |           |                                  | 82,7544   | 84,005    | 85,721    |

Table 10. Determination Results of Methampyrone Content in Jamu Pegal Linu Samples

| Sample | BPOM | Concentration $\pm$ SD (ppm) | Concentration $\pm$ SD (%) |
|--------|------|------------------------------|----------------------------|
| A      | —    | 17,972 $\pm$ 0,011           | 3,594 $\pm$ 0,002          |
| B      | ✓    | 7,537 $\pm$ 0,014            | 1,507 $\pm$ 0,003          |
| N      | —    | 6,931 $\pm$ 0,015            | 1,386 $\pm$ 0,003          |
| I      | ✓    | 14,498 $\pm$ 0,014           | 2,900 $\pm$ 0,003          |
| L      | —    | 15,935 $\pm$ 0,014           | 31,870 $\pm$ 0,028         |

### Qualitative Analysis Using FTIR Spectrophotometry

FTIR (Fourier Transform Infrared) spectroscopy is a chemical analysis technique that is very effective for identifying and recognizing the atomic or molecular structure of a compound (Permatasari et al., 2021). Qualitative analysis was carried out using FTIR-ATR spectrophotometry by looking at the functional groups of the identified compounds, namely methampyrone in the herbal medicine samples for stiffness. FTIR (Fourier Transform Infrared) spectroscopy was chosen in this study as a qualitative analysis method because it is considered environmentally friendly, fast, and capable of analyzing complex samples without requiring the use of solvents in the process.

Based on standard testing of methampyrone using the FTIR spectrum, functional groups were found at certain wave numbers, including C=O (amide), C=C (alkene), C=C (aromatic), C-H, O=S=O, C-O, S=O, and C-H out-of-plane (alkene) (figure and table 1). These results are in accordance with previous findings namely at 1.656, 1.625, 1.172, 1.152, and 1.047  $\text{cm}^{-1}$  which represent the outer C=O, C=C, O=S=O, CH<sub>2</sub> functional groups plane, and S=O (Fatmarahmi et al., 2022). An overview of the results of FTIR spectrum readings and analysis of five samples of herbal medicine for stiffness (samples A, B, I, L, and N) can be seen in figure 2, figure 3, figure 4, figure 5 and figure 6 as well as table 2, table 3, table 4, table 5, and table 6.

Based on the characterization results between the methampyrone standard and samples A, B, N, I, and L using the FTIR test, it shows that the herbal medicine for stiffness in the five samples (A, B, N, I, and L) probably contains methampyrone. This is proven by comparing the FTIR spectra and descriptions of functional group identification between the methampyrone standard, sample A, sample B, sample N, sample I, and sample L, respectively shown in figure 2, figure 3, figure 4, figure 5, figure 6 above. The five samples obtained functional groups, namely C=O (amide), C=C (alkene), C=C (aromatic), C-H, O=S=O, C-O, S=O, and C-H out-of-plane (alkene). Samples A, N, and L are herbal medicine products that are not registered with BPOM. Samples B and I are registered with BPOM.

### Quantitative Analysis Using UV-Vis Spectrophotometry

Determination of the maximum wavelength was carried out before measuring the absorbance of the concentration of methampyrone standard series solution. Determination of the maximum wavelength was carried out with the aim of obtaining a specific wavelength so that the amount of methampyrone could be absorbed maximally (Gandjar & Rohman, 2012). Measurement at the maximum wavelength ( $\lambda_{\text{max}}$ ) was carried out because at this wavelength, the change in absorbance per unit concentration is the greatest. This means that a small change in the concentration of the substance will produce a significant change in the measured absorbance value, so that the maximum sensitivity of the analysis will be obtained (Apriliyani et al., 2018). Measurement of the maximum wavelength of methampyrone was carried out using a standard solution of methampyrone with a concentration of 100 ppm in the UV wavelength range, namely 200-400 nm. The result of determining the maximum wavelength of methampyrone in 0.1 N HCl solvent is 260.20. HCl 0.1 N solution was chosen as the solvent because methampyrone can dissolve in dilute hydrochloric acid solution (Depkes RI, 2019). In addition, 0.1N HCl solution was used to separate the analyte from the herbal mixture and to remove protein in the sample. The presence of protein in the sample can cause precipitation which can affect the validity of the analysis results (Rusdiana et al., 2020).



This methampyrone calibration curve was made by measuring the absorbance of methampyrone series standard solution with concentrations of 8 ppm, 11 ppm, 14 ppm, 17 ppm, and 20 ppm at a maximum wavelength of 260.20 nm. Based on the measured data of the absorbance value of the methampyrone standard series solution, the calibration curve shows a linear relationship between the concentration of the series standard solution and absorbance, as evidenced by the straight line on the curve (figure 7). The linear regression equation obtained is  $y = 0.0333x + 0.0377$ , with a coefficient of determination ( $R^2$ ) of 0.9997. This regression equation provides data in the form of  $y$  as absorbance,  $x$  as concentration, slope value (slope of the regression line) 0.0333 and intercept value 0.0377.

Linearity is done to ensure that the measured absorbance value is proportional to the levels of test compounds in herbal samples in a certain range linearly. The more linear the calibration curve, the better the method used. The  $R^2$  value obtained was 0.9997. The correlation coefficient value obtained meets the linearity requirement of  $R^2 \geq 0.998$ , which indicates a strong relationship between concentration ( $x$ ) and absorbance ( $y$ ). From the results obtained in this study, the calibration curve is declared to have a linear correlation coefficient (Riyanto, 2014).

LoD can be used to determine whether the concentration of the analyte in the herbal sample is more than or equal to a predetermined limit. The LoD (Limit of Detection) value can be determined by calculating the standard deviation and slope value of the linear regression equation of the methampyrone calibration curve. Based on the calculation, the LoD value obtained was 0.275966 mg/L (table 7). This result indicates that the lowest concentration of methampyrone that can be detected but not quantified is 0.275966 mg/L. If the herbal medicine sample shows a concentration of methampyrone exceeding 0.275966 mg/L, it means that there is methampyrone in the sample. Conversely, if the concentration is below 0.275966 mg/L, then methampyrone is not detected. LoQ (Limit of Quantification) is the lowest concentration of analyte in a sample that can be measured with high accuracy and precision (Taleuzzaman, 2018). As with the LoD, the LoQ value can be determined by calculating based on the standard deviation and slope value of the linear regression equation of the methampyrone calibration curve. Based on the calculation of the research data, the LoQ value obtained was 0.9198867 mg/L (table 7). This result shows that using this method the lowest concentration of methampyrone that can be measured precisely is 0.9198867 mg/L. If the concentration of methampyrone in the sample is below the LoQ value, then the measurement results cannot be considered accurate and precise, and cannot be said to be well quantified.

Precision tests are carried out by making repeated measurements using analytical methods that produce Relative Standard Deviation (RSD) values. The precision test in this study was carried out using repeatability analysis, namely analysis carried out using analytical procedures in the laboratory in a short time with the same analyst with the same equipment (Indrayanto, 2022). The precision test results listed in table 4.10 show that the %RSD values for 6 repetitions of each herbal medicine sample A, B, N, I, and L at a maximum wavelength of 260.20 nm are as follows: 0.0682%; 0.2058%; 0.2373%; 0.1134%; and 0.0973%, with an average of 0.1444% (table 8). The %RSD values of the five samples are below 1%, indicating that this method has a very high level of precision for testing herbal medicine samples at these wavelengths.

The accuracy test in this study was carried out using the standard addition method with three concentration levels and 3 repetitions (Chavan & Dasai, 2022). The concentration of methampyrone standard added in the sample was 8 ppm, 14 ppm, and 20 ppm. Furthermore, the standard addition solution was measured using UV-Vis spectrophotometry at a maximum wavelength of 260.20 nm, then the percentage of recovery was calculated by the difference between the results of the two measurements compared to the expected results. Table 9 shows that the absorbance of the sample solution added to the standard is higher than the absorbance of the sample. The percentage value of the recovery of the jamu pegal linu sample that has been added to the methampyrone standard solution with a concentration of 8 ppm, 14 ppm, and 20 ppm can be seen in table 9. The average recovery percentage is 82.7544%; 84.005%; and 85.721%. Ideally, all analytes added to the sample should be recovered without loss of analytes, thus achieving a recovery percentage of 100%. A decrease in the percentage of recovery indicates the loss of analytes (Kantasubara, 2015). The ideal percentage recovery is quite difficult to obtain due to the possibility of high sensitivity to interference in complex sample matrices, especially when the analyte has a very low concentration (mg/L) (Ebdon et al., 1998). The range of percentage recovery results of the five herbal medicine samples for analyte concentrations of 8-20 ppm was 82.7544% to 85.721%. These results fall into the required range of 80-120% (Rohman, 2016). The values obtained prove that the UV-Vis spectrophotometric method is accurate as a method of analyzing methampyrone in herbal medicine samples and can be used for this study.

The determination of levels is carried out by first preparing the sample and diluting the sample. Samples A, B, N, and I were diluted 2 times each and sample L was diluted 20 times. Table 10 shows that the levels of methampyrone (ppm) in samples A, B, N, I, and L exceeded the LoD value of 0.2389936 ppm and LoQ value of 0.7966452 ppm based on the calculation of methampyrone levels. Methampyrone in the jamu pegal linu samples can be detected and quantified. The levels of samples A, B, N, I, L were  $17.972 \pm 0.011$  ppm;  $7.537 \pm 0.014$  ppm;  $6.931 \pm 0.015$  ppm;  $14.498 \pm 0.014$  ppm; and  $15.935 \pm 0.014$  ppm, respectively. The calculation of methampyrone content in jamu pegal linu samples continued until it was obtained in percent form. The test results showed that samples A, B, N, I, and L had

methampyrone levels of  $3.594 \pm 0.002\%$ ;  $1.507 \pm 0.003\%$ ;  $1.386 \pm 0.003\%$ ;  $2.900 \pm 0.003\%$ ; and  $31.870 \pm 0.028\%$ , respectively. Samples A, N, and L included the distribution license number but were not registered with the BPOM, while samples B and I included the distribution license number and were registered with the BPOM.

## CONCLUSION

The FTIR spectra formed on the five samples show the wave numbers of the functional groups C=O (amide), C=C (aromatic), C-H, O=S=O, C-O, S=O, and C-H out-of-plane (alkene), which are the functional groups of methampyrone.

Validation of the analytical method using UV-Vis Spectrophotometry demonstrates its suitability for quantifying methampyrone levels in jamu pegal linu. The method validation parameters include a correlation coefficient ( $R^2$ ) of 0.9997; limit of detection (LOD) of 0.275966 mg/L; limit of quantification (LOQ) of 0.9198867 mg/L; precision expressed as % RSD of 0.1444%; and accuracy indicated by %recovery of 82,7544%; 84,005%; and 85,721%. The results from the validated analysis method reveal the presence of methampyrone in jamu pegal linu across the five samples (A, B, N, I, and L), with concentrations in each sample measured at  $3,594 \pm 0,002\%$ ;  $1,507 \pm 0,003\%$ ;  $1,386 \pm 0,003\%$ ;  $2,900 \pm 0,003\%$ ; and  $31,870 \pm 0,028\%$ .

## ACKNOWLEDGMENTS

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in this research.

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