LUX MENSANA **Volume 3, Issue 3, 2024**
Page. 343-353

Original Research Article

ANALYSIS OF METANIL YELLOW IN PACKAGED JAMU BY UV-VIS SPECTROPHOTOMETRIC METHOD

Sri Lestari[1)*], Intan Ayu Kusuma Pramushinta[1)], Amanda Safithri Sinulingga[1)]
[1)] Department of Pharmacy, Faculty of Health Sciences, Universitas PGRI Adi Buana Surabaya
*Corresponding Author, E-mail: srilestari13102001@gmail.com

ABSTRACT

Introduction. Metanil yellow is a synthetic coloring material used for textile products, wood paint, painting paint, and fur. Despite its use having been banned in the Regulation of the Minister of Health of Indonesia No. 239/MENKES/PER/V/1985, metanil yellow is still commonly found in the local community. In Indonesia, this coloring material is often misused to color various types of food, including herbs, crackers, noodles, tofu, and yellow snacks. The purpose of this research is to validate the analytical method for determining metanil yellow levels in herbal medicine of jamu using the UV-Vis spectrophotometric method. **Method.** In this research, the determination of metanil yellow levels was carried out by instrument analysis using the UV-Vis Sectrophotometric method. **Result & Analysis.** In the linearity validation parameter, the regression equation y = 0.0548x-0.01398 is obtained with an r^2 value of 0.9999, the LOD parameter is 0.11686 ppm, LOQ is 0.5620 ppm, the precision parameter RSD value obtained is 0.87% which meets the requirements of ≤ 4%. Furthermore, the results on the determination of metanil yellow levels in each sample A obtained a percentage of 0.00006755% ± 3.9260; sample B obtained a percentage of 0.000110% ± 10.5309; and sample C obtained a percentage of 0.0001023% ± 9.2998. **Discussion.** Given the very low concentration, it is possible that the yellow color in the herbal medicine samples is not completely caused by metanil yellow, but it might also be influenced by other natural ingredients with yellow pigments.

Keywords: Metanil Yellow, Jamu, UV-Vis Spectrophotometry, Analytical Method Validation

INTRODUCTION

Indonesia has traditional medicine techniques that have been inherited and transmitted through generations. Traditional medicine is typically perceived as considerably safer than contemporary medicine as it is less potent, cheaper, and more convenient. *Jamu* is a widely recognized traditional herbal medicine used by the community to treat minor ailments, prevent disease, and maintain overall health and immune system function (Kusumo *et al.*, 2020).

Jamu is typically composed of botanical constituents obtained from plants, such as roots, leaves, flowers, and tree bark. Jamu, as a popular traditional medicine, plays an important role in providing healthcare services to people in less developed countries. An estimated 70-80% of the population in developing countries rely on traditional medicine, as per estimates. Jamu is a concoction based on the roots of various plants, including turmeric, ginger, galangal, curcuma and many others. In addition, jamu also incorporates several types of leaves, such as bay leaves, betel leaves, tamarind leaves and many more. The making of jamu is usually by directly harvesting from nature and then processed without the use of artificial chemicals. This difference lies in the utilization of herbs or plants in traditional medicine, as opposed to contemporary medicine. This processing includes extraction of natural elements by boiling, water separation, and consumption of the finished product. Over time, herbal medicine processing techniques have progressed significantly. Today, jamu is usually made into pills, capsules, caplets, or liquids. Complementary and alternative medicine are commonly used terms to describe this (Azizuddin, 2021).

According to research from the Research and Development Agency of the Ministry of Health of Indonesia, half of Indonesia's population considers jamu as a traditional medicine. Half of Indonesians use jamu for preventive health measures and to treat diseases (Agustanti, Restyana and Savitri, 2021). The data indicates that jamu, which is one component of traditional medicine, has been used by the Indonesian population. Jamu sellers typically offer a variety of herbs, such as *kunir asam*, *brotowali*, *kencur* rice, *sinom*, *puyang* chili, betel, ginger, kecutan, pahitan and *uyup-uyup* (Prabawa and Fitriani, 2020).

Traditional medicine refers to the use of plant, animal, mineral, or a combination of these materials, either in their natural form or processed in a certain way (such as galenics), which have been used for medicinal purposes through generations on the basis of accumulated knowledge and experience (Kumontoy, Deeng and Mulianti, 2023). Jamu is a traditional Indonesian herbal medicine that has been used for generations by Indonesians to boost health and relieve disease. Jamu is particularly popular in rural and urban areas, despite the increasing dominance of modern medicine (Elfahmi, Woerdenbag and Kayser, 2014). Irresponsible industries in Indonesia are exploiting the growing public interest in herbal medicine and the competitiveness of the industry by introducing banned ingredients and medicinal chemicals (BKO) into herbal products (Sahumena *et al.*, 2020).

Traditional herbal medicine, which is an essential aspect of Indonesia's cultural heritage, is a widely used substitute for mainstream medicine. Herbal products should avoid the use of animal-derived

ingredients, such as goat bile or crocodile stems. Jamu usually comes with a bitter taste, requiring the use of honey as a sweetener to enhance the flavor (Winarno, 2021). In accordance with Regulation of the Minister of Health of Indonesia No. 003/MENKES/PER/I/2010, jamu is prohibited from containing synthetic colorants due to its chemical nature.

Herbs are tainted with artificial dyes, particularly those intended for textiles and leather, which are then incorporated into food. Metanil yellow is a prohibited pigment that is often used in fabric dyeing or skin care. However, it is often misused in various food products despite its prohibition (Indrawati and Mutmainnah, 2022).

Metanil yellow is a synthetic pigment in a brownish yellow powder. It is soluble in water and alcohol, and to a lesser extent in benzene and ether. It has low solubility in acetone. This colorant is often used as a pigment in textiles, paper, ink, plastics, leather, and paint, and serves as an acid-base indicator in the laboratory. However, in Indonesia this colorant is often misused to color various culinary products, such as packaged herbs, crackers, noodles, tofu, and yellow snacks. Metanil yellow is prohibited from being used in food as it may potentially irritate the digestive tract when it is ingested. In addition, this substance can cause symptoms such as nausea, vomiting, abdominal pain, diarrhea, fever, weakness and hypotension (Zulkifli, Andina and Primanadini, 2017).

Metanil yellow is a textile coloring often used improperly as a food coloring product. Nowadays, metanil yellow is used improperly in food products, especially in products such as crackers, noodles, fried yellow snacks and is also used as a coloring material in tofu.

A previous research found that there was metanil yellow in breadcrumbs being sold in traditional markets in Makassar (Sahani and Juliani, 2019). Out of 14 samples of breadcrumbs were positive for containing metanil yellow with a percentage of 35.7%. The misuse of metanil yellow as a food coloring may be caused by public ignorance regarding the proper coloring materials for food as well as the lack of labels that explicitly prohibit the use of these compounds in food. In addition, the relatively cheaper price of coloring products for industrial use compared to coloring products specifically intended for food may also contribute to this problem. Textile colorings exhibit vibrant colors and offer convenience in application. These colors are easily accessible in compact packaging, thus allowing affordability for individuals of lower socioeconomic status (Singapurwa, 2022).

Considering the potential dangers, it is necessary to test metanil yellow in packaged jamu using certain analytical methods. The analytical method that can be used to analyze metanil yellow is UV-Vis Spectrophotometry (Nugraha, Kurniawan and Yastiara, 2023).

The aim of this research is to validate the method of determining metanil yellow levels using UV-Vis Spectrophotometry, which includes linearity, LOD, LOQ, precision, and selectivity criteria, and determine the amount of metanil yellow as a coloring ingredient in packaged jamu using the UV-Vis Spectrophotometry method.

METHOD AND ANALYSIS

This is a quantitative research using laboratory experimental method. Methods commonly used to analyze metanil yellow

are UV-Vis spectrophotometry, HPLC, High Performance Liquid Chromatography, and FTIR.

This research applied UV-Vis Spectrophotometry method to determine the metanil yellow concentration of a compound that contains chromophore and auxochrome functional groups.

This research aims to analyze the metanil yellow in packaged jamu, with the independent variable is jamu packaged and the dependent variable is the content of metanil yellow. The research was conducted from January to April 2024 at the Pharmaceutical Chemistry Laboratory, Faculty of Health Science, PGRI AdiBuana University Surabaya. The materials used include samples of jamu (both registered with the Food and Drug Administration (BPOM) and non BPOM), distilled water, HCl 2N, and metanil yellow. The tools used include beaker glass, glass funnel, drop pipette, filter paper, micropipette, analytical balance, and UV-Vis spectrophotometry.

RESULT

Tests were carried out on three samples of jamu obtained in Gayungan, Surabaya. The determination of levels was carried out by UV-Vis spectrophotometric method which was previously validated first. The purpose of this validation is to ensure that an analytical method produces accurate, specific and effective analytical results. The main objective of method validation is to produce the optimal analytical results (Maghfiroh, Monica and Afthoni, 2022).

Determination of the Maximum Wavelength

The purpose of the maximum wavelength is to determine the absorption area that can be produced by the absorbance value (Sukmawati, 2018). Determination of the maximum wavelength in this research was carried out on 100 ppm base solution.

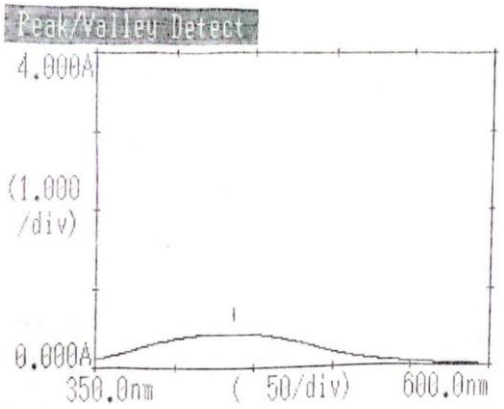


Figure 1. Maximum Wavelength of Metanil Yellow

The maximum wavelength obtained in this research was 437 nm with an absorbance value of 0.8942.

Method Validation

Method validation is an essential step in proving the quality of a quantitative test. Method validation was carried out due to differences in place, time and samples used in this research that were different from other studies. This research was also carried out with different tools and analysts therefore validation is required to ensure the quality of the methods carried out. Validation aims to prove that the results obtained are valid to meet the requirements of their use (Rohmah, Muadifah and Martha, 2021). This research includes validation tests which include linearity, limit of detection (LOD), limit of quantification (LOQ), precision and selectivity.

Linearity

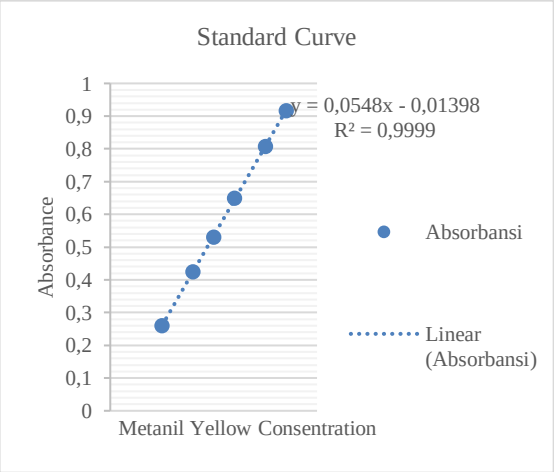
The purpose of the linearity test is to determine whether there is a linear or non-linear relationship between the tested

substance and the UV-Vis spectrophotometric method (Wardhani and Nurbayanti, 2019). The higher the concentration of the analyte, the higher the absorbance value of the analyte (Anngela, Muadifah and Nugraha, 2021). Based on Table 1, the absorbance results are higher as the concentration of metanil yellow standard solution is higher. The relationship between concentration and absorbance is represented in the calibration curve.

Table 1. Absorbance of Standard Curve

Concentration (ppm)	Absorbance
5	0.2604
8	0.4239
10	0.5306
12	0.6489
15	0.8073
17	0.9167

Source: Processed Data by Researchers



Graph 1. Linearity Standard Curve

The results of the absorbance data obtained a linear regression equation y = 0.0548x - 0.01398, with a value of r^2 = 0.9999. A correlation coefficient value approaching 1 indicates a strong linear relationship between absorbance and analyte concentration (Chakti, Simaremare and Pratiwi, 2019). The correlation coefficient r obtained in this research is considered satisfactory as it is approaching 1. An increase in the linear line on the curve can indicate a linear relationship between concentration and absorbance (Anngela, Muadifah and Nugraha, 2021). It can be seen in the graph 1 that the linear line has increased, suggesting that the analysis of metanil yellow using UV-Vis spectrophotometry is of good linearity.

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

Limit of Detection (LOD) is the smallest level of analyte in a sample that can still be detected by a device. Limit of Quantitation (LOQ) is the lowest concentration of analyte in a sample that can be measured quantitatively with acceptable accuracy and precision (Harmono, 2020).

The test results of limit of detection (LOD) and limit of quantitation (LOQ) resulted in a regression equation y = 0.0548x - 0.0139 with a value of r^2 = 0.9999. The test results of limit of detection (LOD) and limit of quantitation (LOQ) can be seen in Table 2.

Table 2. LOQ and LOQ Data

Cons (ppm)	Abs
5	0.2604
8	0.4239
10	05306
12	0.6489
15	0.8073
17	0.9167
SD Value	0.00308
LOD	0.1686 ppm
LOQ	0.5620 ppm

Source: Processed Data by Researchers

LOD and LOQ were calculated by measuring the absorbance of standard solutions with concentrations of 5 ppm, 8 ppm, 10 ppm, 12 ppm, 15 ppm, and 17 ppm.

The slope value of the regression equation and Sy were used to determine the limit of detection and limit of quantification. The limit of detection was determined by multiplying the Sy value by 3. For the calculation of the limit of quantitation, the same procedure was practiced, except that the Sy value was multiplied by 10. The obtained LOD value of 0.1686 ppm indicates that sample measurements can still be done at that concentration, giving accurate results based on the individual accuracy of the analysis. Similarly, the LOQ value of 0.5620 ppm indicates that accurate analysis can still be achieved when measurements are taken at that concentration (Wardani, 2022). This is the smallest concentration or amount of analyte in the sample that can be measured precisely by the instrument (Ramadhan and Musrifoh, 2021).

Precision

Precision is a measurement that shows conformity between test results that are applied repeatedly (repeatability) (Harmono, 2020). The calculation results show data on Standard Deviation (SD) and Relative Standard Deviation (RSD). The precision results are presented in Table 3 below.

Table 3. Results of Precision Test

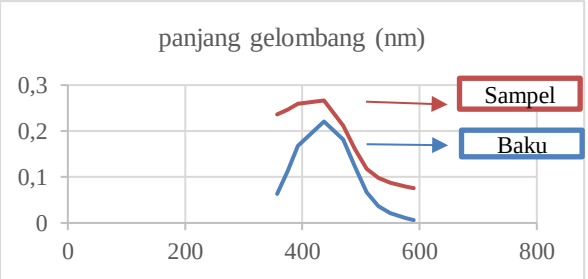
Replication	Absorbance	Level (ppm)
1	0.2427	4.66
2	0.2437	4.7
3	0.2428	4.68
4	0.2444	4.71
5	0.2440	4.7
6	0.2484	4.78
Average		4.7
SD Value		0.0412
RSD Value %		0.87 %

Source: Processed Data by Researchers

Referring to Table 3, a precision test was carried out with the coefficient of variation (RSD) value obtained of 0.87%. The value obtained meets the criteria for precision test requirements, which is ≤ 4% (Iman, Auli and Sukrasno, 2023). It can be concluded that in this research the value of the coefficient of variation (RSD) obtained has met the requirements.

Selectivity

The purpose of the selectivity test is to determine changes in the shape of the curve and the wavelength shift of metanil yellow caused by the entry of chemicals in the sample (Sayuthi and Kurniawati, 2017). The measurement results of the standard sample and the test sample obtained from the selectivity determination is presented in Graph 2.



Graph 2. Measurement Results of Standards and Samples Wavelength 350-600

Graph 2 illustrates that the metanil yellow standard has a wavelength of 437 nm with an absorbance of 0.2208 while the sample also has a wavelength of 437 nm with an absorbance of 0.2666. Both show identical spectral peaks, indicating that the analytical approach has excellent selectivity at the specific wavelength of 437 nm (Syahruni and Arrisujaya, 2019).

DISCUSSION

Determination of metanil yellow levels in this research was carried out using the UV-Vis spectrophotometric method with a wavelength of 437 using a standard solution of metanil yellow. The results of the regression equation y = 0.0548x - 0.01398) with a r^2 value of 0.9999. The results of sample measurements in this research can be seen in Table 4.

Table 4. Sample Measurement Results

Sample	Replication	Absorbance	Concentration (ppm)	Average (ppm) ± SD	Concentration % b/v
A	1	0.1431	2.8649	6.7541 ± 3.9260	0.00006755
	2	0.1461	2.9197		
	3	0.1455	2.9087		
B	1	0.2581	4.9635	11.6555 ± 10.5309	0.00001190
	2	0.2616	5.0273		
	3	0.2598	4.9945		
C	1	0.2253	4.3649	10..2304 ± 9.2998	0.0001023
	2	0.2268	4.3923		
	3	0.2283	4.4197		

Source: Processed Data by Researchers

50% of the Indonesian population uses jamu for preventive health purposes and treatment of diseases (Maharianingsih, 2023). The data indicates that jamu is one of the components of traditional medicine that has been widely used by Indonesians (Isnawati and Sumarno, 2021). According to the Regulation of the Minister of Health of Indonesia No. 239/MENKES/PER/V/1985, metanil yellow has been classified as a hazardous substance and is prohibited for use. Metanil yellow is exclusively permitted for coloring wool, nylon, leather, paper, paint, metal, detergent, wood, fur and cosmetics (Simanjuntak, 2020).

Data collected from samples A, B and C that were sold to the public indicated that the levels of metanil yellow obtained were 0.00006755%, 0.00011902% and 0.0001023%. According to Zulkifli, Andina and Primanadini (2017), metanil yellow should never be used. Given the values obtained, considering the very low levels, there is a possibility of yellow color in the sample.

Metanil yellow coloring material poses significant hazards if inhaled, exposed to the skin, in contact with the eyes, or swallowed. Effects include respiratory tract irritation, skin irritation, eye irritation, and increased risk of bladder and urinary tract cancer. Ingesting this substance may cause irritation of the gastrointestinal tract, feelings of nausea, vomiting, abdominal discomfort, diarrhea, increased body temperature, weakness, and decreased blood pressure (Aini, 2019).

CONCLUSION AND SUGGESTION

Conclusion

From the research that has been conducted, it can be concluded that the validation testing of analytical methods in the determination of metanil yellow concentration meets the criteria for validation of analytical methods in UV-Vis spectrophotometry with linear regression results y = 0.0548x-0.01398 with an r^2 value of 0.9999, LOD parameters 0.1686 ppm, LOQ 0.5620 ppm, precision parameters with an RSD value of 0.87% which meets the requirements of ≤ 4%, and selectivity of raw and samples have similar peak points at a wavelength of 437 nm, making the method used in this research selective. The concentration of metanil yellow in jamu samples that have been carried out using UV-Vis

spectrophotometry for each sample A obtained a percentage of 0.00006755% ± 3.9260; sample B obtained a percentage of 0.0001190% ± 10.5390; and sample C obtained a percentage of 0.0001023% ± 9.2998.

Suggestion

Further research is necessary by using various additional instruments such as HPLC, KLT, KCKT, and FTIR to compare the results obtained by the UV-Vis spectrophotometric method. This multi-instrument approach will not only increase the validity and reliability of the results but also provide a more comprehensive picture of the metanil yellow in the packaged jamu. Each analytical technique has its own advantages, thus by combining several methods, it is expected that the results obtained will be more accurate and in-depth, and can be used as a basis for the development of better research and regulatory implementation in the future.

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