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Validation of Methylene Blue Analysis Method in Wastewater Samples by UV-Vis Spectrophotometry

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ABSTRACT. Methylene blue (MB) is an extensively utilized cationic dye in the textile industry. MB is, however, a highly noxious substance that can have detrimental effects on both the environment and human health. MB can pollute waterways and cause the mortality of aquatic organisms in the environment. Due to the hazards posed by MB, it is crucial to have an effective method for analyzing its concentration in wastewater. This will ensure that MB-contaminated water is appropriately treated and disposed of, thereby protecting the environment and human health. One of the analyses utilized the Evolution 360 UV-Vis Spectrophotometer to determine methylene blue concentrations. The Evolution 360 UV-Vis Spectrophotometer method was validated by measuring linearity tests, limit of detection and quantification, precision, and accuracy. The R-value for linearity measurements is greater than 0.99, indicating that the method is proportionally validated. From this research, the LOD and LOQ values were 0.46415 mg/kg and 1.54716 mg/kg, respectively. As required, the precision measurement yields acceptable results, with a %RSD value of less than 2%, and the accuracy measurement yields a recovery of 100% (between 80 and 110%). So that the method for measuring the concentration of methylene blue in water using the UV-Vis Evolution 360 Spectrophotometer satisfies the requirements for linearity, precision, and accuracy.

1. INTRODUCTION

The development of the textile industry has the potential to cause environmental problems, especially water pollution caused by the excessive use of textile dyes. Until now, there are more than 100,000 types of commercial dyes used in the textile industry [1,2]. Most textile dyes are synthetic dyes, which are made from chemicals that are not naturally found in the environment. These dyes are often toxic to aquatic life and can also be harmful to human health. Synthetic dyes are light-stable, meaning they do not degrade quickly when exposed to sunlight. This makes them difficult to remove from water bodies, and they can persist in the environment for long periods of time. When synthetic dyes are released into aquatic systems, they can cause a variety of detrimental effects. They can reduce light penetration, which can affect the photosynthetic activity of aquatic vegetation. This can reduce the amount of oxygen and nutrients available to aquatic organisms, ultimately leading to their demise. Additionally, synthetic dyes can be directly deleterious to aquatic organisms. They can compromise the immune system, cause DNA damage, and even cause cancer. The presence of synthetic dyes in aquatic systems poses a significant environmental threat [3].

Methylene blue (MB) is a synthetic dye that is widely used in the textile industry, especially in the process of dyeing wool and cotton. In addition, MB is also commonly used as a fungicide in aquariums and cosmetics [4]. During the textile dyeing process, approximately 10 to 15 percent of the dyes used are discharged into the wastewater, contributing to environmental contamination [5]. Exposure to high doses of methylene blue, which is a carcinogen, when ingested or consumed by humans can have negative effects on health such as increased heart rate, nausea, abdominal and chest pain, shock, cyanotic jaundice, cerebral palsy, and hypertension [6].

The maximum concentration of MB dye in aquatic systems has been regulated in the Decree of the Minister of Environment and Forestry of the Republic of Indonesia regarding the domestic wastewater quality standard (Number P.68//MENLHK/Setjen/Kum.1/8/2016), around 5.0-10.0mg/L. Thus, related industries need to carry out water treatment and analysis of levels of methylene blue contamination before being discharged into the environment or water bodies. The analysis of MB contamination in water is an important step in ensuring the

quality of water bodies.

The analysis of methylene blue necessitates an exact and reliable method. There are several methods that can be used to analyze MB contamination, including chromatography, electroanalysis and spectrophotometry. The method that is used will depend on the specific application. For example, UV-Vis spectroscopy is a good choice for routine analysis, while HPLC or GC may be required for more sensitive measurements. One of the most widely used analytical methods is UV-Vis spectrophotometry, which utilizes ultraviolet and visible light to measure the absorbance of a chemical compound in a sample. Where the absorbance value of the light that is passed will be proportional to the concentration of the solution in the cuvette [7]. However, before this method can be used, it is necessary to validate the analytical method first. Validation of analytical methods is an important process to ensure the accuracy and reliability of measurement results, as well as to minimize errors in the measurement process.

The purpose of this study is to validate the methylene blue analysis method with the UV-Vis Evolution 360 Spectrophotometer on water samples. This research was conducted so that it could provide information related to the validation of analytical methods with UV-Vis Evolution 360 Spectrophotometry to determine the level of methylene blue in a sample. The stages of the experiment were to make a standard methylene blue solution with a certain concentration, then determine the maximum wavelength based on the UV-Vis spectrum, make a calibration curve of methylene blue, and determine the concentration of methylene blue. The validation stages include linearity testing, measuring the accuracy and precision of the method, determining the limit of detection (LOD) and limit of quantification (LOQ).

2. MATERIALS AND METHODS

The experimental stages included preparation of the equipment and materials used, preparation of the solution, sample preparation, and validation of the analytical method. Tests carried out include accuracy, precision, LOD and LOQ, as well as uncertainty. The experiment was carried out in the research laboratory of the Department of Chemical Engineering, Bandung State Polytechnic.

2.1 Equipment and Materials

The equipment used in this study included general glassware in chemical laboratories, analytical balance, hotplate stirrer, and UV-Vis Evolution 360 spectrophotometer. The materials used are Methylene Blue ($C_{16}H_{18}N_3SCl$) from Merck, and distilled water.

2.2 Preparation of Methylen Blue Solution

The solution of 100 mg/L methylene blue was prepared using distilled water as a solvent, which was weighed as much as 0.5 g of methylene blue powder dissolved with distilled water in a 100 mL beaker. The solution was transferred to a 500 mL measuring flask and then added with distilled water up to the mark and then homogenized. The solution was stored in a closed brown container until it was used.

2.3 Maximum Wavelength Determination

The absorbance of a standard methylene blue solution with a concentration of 6 mg/L was measured in the UV-Visible wave range of 350-800 nm with a UV-Visible Evolution 360 spectrophotometer. The maximum wavelength of methylene blue is used in subsequent measurements to create a calibration curve for a standard methylene blue solution, linearity, precision, accuracy, LOD, and LOQ.

2.4 Preparation of Calibration curve of Methylene Blue

As much as 0.5; 1; 1.5; 2; and 2.5 mL of a standard solution of 100 mg/L methylene blue was pipetted and then put into a 25 mL measuring flask and diluted using distilled water up to the mark to make a series of standard solutions 2; 4; 6; 8; and 10 mg/L. The absorbance of the methylene blue standard series solution was measured at the maximum wavelength of methylene blue using a UV-Visible Evolution 360 spectrophotometer. The methylene blue calibration curve was used to obtain sample concentration and determine precision, accuracy, uncertainty, LOD, and LOQ.

2.5 Precision Determination

Samples were prepared by pipetting 1.5 mL of methylene blue solution with a concentration of 100 mg/L and placed into a 25 mL measuring flask with distilled water as a solvent. The absorption of the solution was analyzed using a UV-Visible Evolution 360 spectrophotometer at maximum wavelength. The treatment was repeated 6 times from sample preparation.

2.6 Determination of The Accuracy and %Recovery

Samples were prepared by pipetting as much as 1.5 mL of 100 mg/L methylene blue solution in a 25 mL measuring flask and adjusted using distilled water and then homogenized. Accuracy was determined by the standard addition method, namely by adding 1 mL of a standard methylene blue solution with a concentration of 50 mg/L to the sample that had been prepared. Then homogenize the solution and measure the absorbance. The treatment was repeated 6 times from sample preparation.

3. RESULTS AND DISCUSSION

3.1 Linearity Test

Linearity is a key parameter in the validation of analytical methods which states the ability of the analytical method to provide results that are proportional to the concentration of the analyte in the sample (through a clear mathematical transformation with a known concentration range) [8,9]. Linearity is assessed by preparing a series of standard solutions with known concentrations of the analyte. The method is then used to measure the concentration of each standard solution. The results are then plotted as a graph of concentration versus response. In this experiment, the Linearity test was carried out by measuring standard methylene blue solutions with concentrations of 2, 4, 6, 8, and 10 mg/L at the maximum wavelength of methylene blue, which is 664 nm. The absorbance data from the measurement results of the standard methylene blue solution are presented in Table 1, while the standard solution calibration curve is presented in Figure 1.

Table 1. Measurement of the absorbance of a standard methylene blue solution

No.	Sample	Concentration (mg/L)	Absorbance
1	Blank	0	0.000
2	Standard 1	2	0.103
3	Standard 2	4	0.224
4	Standard 3	6	0.324
5	Standard 4	8	0.423
6	Standard 5	10	0.552

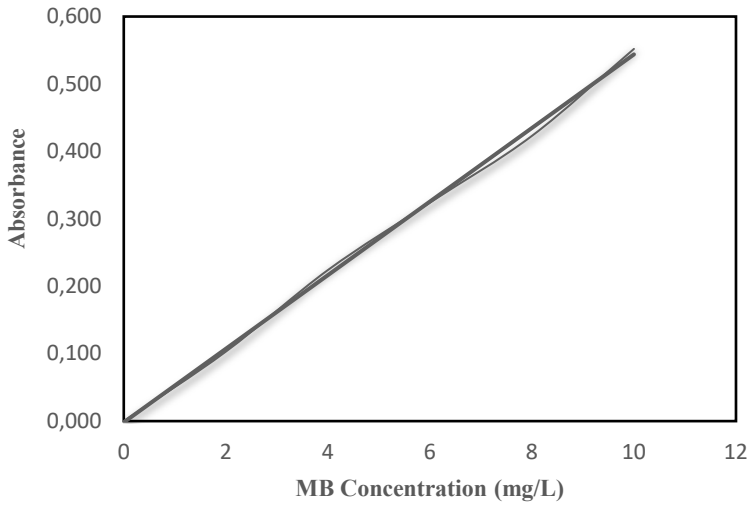

Figure 1. Methylene blue standard solution calibration curve

Based on these data, a linear regression equation Y = 0.0546x - 0.0019 is obtained with a correlation coefficient (R) of 0.9992 and a coefficient of determination (R^2) of 0.9986 so that the linearity of the standard solution with a range of 2-10 mg/L is more than 99%. A correlation coefficient of 1 indicates a perfect linear relationship, while a correlation coefficient of 0 indicates no linear relationship. A correlation coefficient of at least 0.95 is generally considered to be acceptable for most analytical methods. By having a correlation coefficient ≥ 0.99, the calibration curve meets the requirements of linearity acceptance so that the test results on the standard solution used are proportional to the concentration of analytes in the sample working in the range or linear area (2-10 mg/L) [8,9].

3.2 Determining LOD and LOQ

The determination of the LOD and LOQ values was determined to meet the limit test parameters of an analytical method based on linear regression obtained from the absorbance measurements of standard series solutions. Table 2 shows the absorbance values from the measurement results (yi) and the absorbance values that have been corrected (yc) according to the results of the linear regression equation from the calibration curve which are then used to calculate the residual standard deviation.

The limit of detection (LOD) and the limit of quantification (LOQ) are two important parameters that indicate the sensitivity of an analytical method. The LOD is the smallest concentration of an analyte that can be reliably detected, while the LOQ is the lowest concentration of an analyte that can be reliably quantified and meets the parameters of accuracy and thoroughness [10]. From the results of the measurements that have been carried out, the LOD and LOQ values were 0.46415 mg/kg and 1.54716 mg/kg, respectively. So it can be said that the determination of methylene blue in water solvents using a UV-Vis Evolution 360 spectrophotometer can be detected with a significant response.

Table 2. LOD and LOQ determination data

No.	Concentration (mg/L)	Absorbance (yi)	Corrected Absorbance (yc)	$(yi-yc)^2$
1	0	0.000	0.002	0.000003
2	2	0.103	0.107	0.000018
3	4	0.224	0.216	0.000057
4	6	0.324	0.326	0.000002
5	8	0.423	0.435	0.000137
6	10	0.552	0.544	0.000066
Residual standard deviation				0.008443
LOD (mg/kg)				0.464149
LOQ (mg/kg)				1.547162

3.3 Precision Determination

Precision, or the degree of agreement between individual test results obtained repeatedly on the same sample, is a measure of the suitability of an analytical method [9,11,12]. Precision is measured as standard deviation or relative standard deviation (coefficient of variation), which is expressed as repeatability or reproducibility. In the validation of the test method, repeatability was used, which is the precision of the method when performed by the same analyst under the same conditions in a short time interval.

Table 3. Precision determination data

Parameter	Result	Acceptance
Mean	6.2484	
Standard deviation	0.040342	
Relative standard deviation (% RSD)	0.65%	≤ 2.0%

The data presented in Table 3 were obtained from measurements of the sample 6 times under the same conditions and a short time. Table 3 shows the relative standard deviation expressed in % RSD of the methylene blue determination using a UV-Visible Evolution 360 spectrophotometer was 0.65%, which is less than the 2%

acceptance criterion for precision. This indicates that the determination of methylene blue solution in water using UV-Visible Evolution 360 spectrophotometer has good precision.

3.4 Accuracy Determination

Accuracy is a parameter that indicates the degree of closeness of the results of the analysis to the actual analyte concentration. It is expressed as the percent recovery of the analyte added to the sample [9,11,12]. The standard addition method is often used to determine the accuracy of an analytical method. The results of determining the accuracy of the methylene blue determination by spiking the sample with 1 mL of 50 ppm methylene blue standard solution are presented in Table 4.

Table 4. Accuracy determination data

No.	Spike sample	% Recovery
1	1	81,7
2	2	98,5
3	3	105,4
4	4	104,4
5	5	103,5
6	6	103,5
	Average	**100**

Table 4 provides information about the percent recovery (% recovery) of 100% where these results are in the range of 80-110% so that the test method using a UV-Visible 360 spectrophotometer is accurate for determining methylene blue levels in water solvents.

3.5 Uncertainity Test

Determining the value of uncertainty is a calculation that needs to be done when conducting an analytical method validation. From the calculation of this uncertainty will be able to identify the error factor or predict the system error mathematically [12]. Table 5. presents uncertainty data for all treatments and procedures involved in the validation of the spectrophotometric methylene blue analysis method that has been carried out with a combined uncertainty value of ± 0.0390. In general, a lower uncertainty value indicates a higher level of precision and reliability in the measurement

Table 5. Determination of uncertainty

No.	Uncertainity	Uncertainty Test
1	Methylene blue molecular weight ($C_{16}H_{18}ClN_3S$)	423.3163 ± 0.0112 g/mol
2	Methylene blue solution 100 ppm	100 ± 0.0231 ppm
3	Sample dilution factor	67 ± 0.6477
4	Spectrophotometer	y = 0.0546x – 0.0019, R^2 = 0.999 ± 0.1671
5	Homogenity and sample precision	± 0.1009 dan ± 0.1060
	Combined uncertainity	**± 0.0390**

4. CONCLUSION

Based on the validation data of the methylene blue analysis method in wastewater using a UV-Vis Evolution 360 spectrophotometer, good linearity, precision and accuracy are acceptable with the smallest assay limit of 0.4642 mg/kg and the quantification test limit of 1.5472 mg /kg.

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