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Article

Analysis of Rhodamine B Content in Lipsticks Available on the Market in the Wonosobo Region Using Rapid Test Kits and UV–Vis Spectrophotometry

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Abstract

Rhodamine B is a synthetic dye commonly used in the textile and paper industries but prohibited in cosmetic products due to its harmful effects on human health. Despite regulatory restrictions, Rhodamine B is still occasionally misused in cosmetics because of its low cost and ability to produce bright and attractive colors. Exposure to Rhodamine B may cause adverse health effects. This study aimed to identify and quantify the presence of Rhodamine B in lipstick products marketed in the Wonosobo region. Qualitative analysis was conducted using a rapid test kit to detect the presence of Rhodamine B based on color changes after reagent addition. Samples suspected of containing Rhodamine B were subsequently analyzed quantitatively using UV–Vis spectrophotometry. The qualitative results showed that 5 out of 17 lipstick samples tested were suspected of containing Rhodamine B, namely samples 4, 5, 11, 13, and 15. Quantitative analysis confirmed Rhodamine B concentrations of 0.1213 mg/g, 0.0861 mg/g, 0.0584 mg/g, 0.0961 mg/g, and 0.1082 mg/g in samples 4, 5, 11, 13, and 15, respectively. These findings indicate that lipsticks suspected of containing hazardous dyes are still circulating in the market, highlighting the need for stricter monitoring and increased public awareness regarding cosmetic safety.

Keywords: Rhodamine B; Lipstick; Rapid Test Kit; UV-Vis Spectrophotometry

1. INTRODUCTION

Cosmetics are substances or preparations intended for application to the external parts of the human body, such as the epidermis, hair, nails, lips, and external genital organs. They may also be applied to the teeth and oral mucosa for the purposes of cleansing, perfuming, altering appearance, correcting body odor, and/or protecting and maintaining the body in good condition [1]. Lipstick is a cosmetic product used to color the lips and generally contains moisturizing agents, colorants, and protective ingredients that help shield the lips from environmental factors that may cause damage. The colorants used in lipstick may be derived from natural or synthetic sources. Despite the benefits provided by many lipstick ingredients, some manufacturers still incorporate hazardous substances, including synthetic dyes that are prohibited for use in lipstick formulations. The use of harmful substances in cosmetic products, despite awareness of their potential adverse health effects, is unjustifiable. Manufacturers are responsible for ensuring that their products are formulated using safe and beneficial ingredients for consumers [2].

One synthetic dye that is prohibited in lipstick products is Rhodamine B. Rhodamine B is a bright red fluorescent dye that is odorless and appears as green or reddish-purple crystalline powder or crystals. It is commonly used as a coloring agent in paper, textile, and paint industries [3]. Long-term exposure to Rhodamine B may cause various adverse health effects, including respiratory tract irritation, eye irritation, skin irritation, nausea, vomiting, liver cancer, and even death when present in cosmetic products containing hazardous substances [4]. The toxicity of Rhodamine B is associated with the presence of chlorine (Cl), a reactive halogen compound. When chlorine enters the body, it tends to form bonds with other compounds in an attempt to achieve stability, potentially disrupting their normal biological functions and impairing physiological processes. In addition, Rhodamine B contains alkylating groups (CH_3–CH_3) that can interact with proteins, lipids, and DNA due to their radical properties [5].

Rhodamine B is a prohibited synthetic dye in cosmetic products due to its toxicity and potential to damage body tissues. Its presence in cosmetics can be detected using UV–Visible spectrophotometry because it contains chromophore groups that absorb ultraviolet and visible light at specific maximum wavelengths [6]. Previous studies investigating Rhodamine B contamination in cosmetic products have generally been conducted in large urban areas and major commercial centers, while information regarding the safety of cosmetic products marketed in smaller districts remains limited. Wonosobo Regency represents a growing market for cosmetic products with diverse distribution channels, including conventional markets, cosmetic stores, and online-based resellers. However, data on the presence of Rhodamine B in lipstick sold in this region are still scarce. Therefore, this study provides novel information on the safety profile of lipsticks marketed in Wonosobo by evaluating the presence and concentration of Rhodamine B using a rapid test kit for qualitative screening and UV–Visible spectrophotometry for quantitative determination. The findings are expected to contribute to regional cosmetic surveillance efforts and provide scientific evidence to support consumer protection against hazardous cosmetic products.

2. MATERIALS AND METHODS

2.1. Material

Rapid test kit Rhodamine B (Labtest), 17 lipstick samples red color without brand and without registered number purchased from three different local markets in Wonosobo, Rhodamine B standard (Merck), HCl 4 N (Merck), Methanol pa (Merck).

2.2. Instrument

An analytical balance (Mettler Toledo), test tubes (Pyrex), glass stirring rods (Pyrex), graduated cylinders (Pyrex), beakers (Pyrex), filter paper (Whatman), 10 mL, 25 mL, and 50 mL volumetric flasks (Pyrex), a micropipette (Socorex), and a double-beam UV–Visible spectrophotometer (JASCO V-730, Japan).

2.3. Method

2.3.1 Qualitative Analysis of Rhodamine B using Rapid Test Kit

Approximately 1 g of lipstick sample was accurately weighed and melted on a hot plate until fully liquefied. Subsequently, the sample was dissolved in 20 mL of analytical-grade methanol (p.a.) and stirred thoroughly to ensure complete dissolution of the colorant components. A test tube was prepared, and 1–3 mL of the sample solution was placed into the tube. Two drops of Reagent 1 from the Rhodamine B rapid test kit were added and mixed, followed by the addition of four drops of Reagent 2. The reaction mixture was allowed to stand for 15–20 minutes. The appearance of a purple color was interpreted as a positive indication of the presence of Rhodamine B in the sample.

2.3.2 Quantitatve Analysis of Rhodamine B Using Spectrophotometry UV-Visible

1. Preparation of Rhodamine B standard

   An accurately weighed 50 mg portion of Rhodamine B standard was transferred into a 50 mL volumetric flask. Methanol was added in a sufficient amount to dissolve the standard, and the solution was mixed until fully homogenized. The volume was subsequently made up to the mark with methanol and thoroughly mixed to obtain a homogeneous stock solution of Rhodamine B.

   An aliquot of 2.5 mL of the 1000 ppm Rhodamine B stock solution was pipetted into a 50 mL volumetric flask. The volume was adjusted to the mark with methanol, then thoroughly mixed to achieve complete homogenization. The resulting standard solution had a concentration of 50 ppm.

2. Determination of Maximum Wavelength (λmax)

   An aliquot of 2 mL of the 50 ppm Rhodamine B standard solution was pipetted into a 50 mL volumetric flask. The volume was adjusted to the mark with methanol and mixed thoroughly to ensure complete homogenization, yielding a Rhodamine B working solution with a concentration of 2 ppm. The absorption spectrum of the solution was subsequently recorded over the wavelength range of 400–800 nm using a UV–Visible spectrophotometer to determine the maximum absorption wavelength (λmax), with methanol serving as the blank. Aliquots of 0.2, 0.3, 0.4, 0.5, 0.6, and 0.7 mL of the 50 ppm Rhodamine B standard solution were accurately pipetted into separate 10 mL volumetric flasks.

3. Evaluation of Calibration Curve Linearity

   The solutions were diluted to volume with methanol and mixed thoroughly to obtain standard solutions with final concentrations of 1.0, 1.5, 2.0, 2.5, 3.0, and 3.5 ppm, respectively. The absorbance of each solution was measured at the previously established maximum absorption wavelength (λmax) using methanol as the blank. A calibration curve was then constructed by plotting absorbance against concentration.

4. Sample Preparation and Measurement

   Positive result sample from qualitative analysis were accurately weighed 1 g sample and placed in a volumetric flask. Eight drops of 4 N hydrochloric acid (HCl) and 10 mL of methanol were added, and the mixture was thoroughly homogenized. The solution was subsequently filtered to obtain a clear filtrate. The filtrate was transferred into a 25 mL volumetric flask, diluted to volume with methanol, and mixed thoroughly. The solution was then measured using a UV–Visible spectrophotometer at the previously determined maximum absorption wavelength (λmax). The absorbance results were plotted into the calibration linearity curve to obtain the concentration value of Rhodamine B in sample.

3. RESULT AND DISCUSSION

This study was conducted using two analytical methods: a qualitative method employing a Rhodamine B rapid test kit and a quantitative method using UV–Visible spectrophotometry. The application of these complementary methods was intended to provide a comprehensive assessment of the presence of Rhodamine B in lipstick samples as well as to determine

its concentration. The rapid test kit was used as a preliminary screening method to detect the presence or absence of Rhodamine B in the samples. Following qualitative analysis, quantitative determination was performed using UV–Visible spectrophotometry to measure the concentration of Rhodamine B in samples that tested positive. The UV–Visible spectrophotometric method operates by measuring the absorbance of sample solutions at a specific wavelength corresponding to the characteristic absorption of the analyte.

A total of 17 lipstick samples were collected from three different markets in Wonosobo Regency. The sampling locations were selected based on field observations indicating the widespread availability of lipstick products lacking official authorization from the Indonesian Food and Drug Authority (BPOM) and without ingredient information on the product packaging. Cosmetic products marketed without proper registration and clear labeling may potentially contain hazardous substances that pose health risks to consumers. Therefore, it is important to investigate the presence and concentration of potentially harmful compounds in lipstick products available on the market.

3.1 Qualitative Analysis of Rhodamine B Using Rapid Test Kit

The rapid test kit consisted of two reagents: Reagent 1 containing antimony pentachloride ($SbCl_5$) in 5 N HCl and Reagent 2 containing toluene (methylbenzene). In samples containing Rhodamine B, the red color remained stable and did not disappear or fade after the addition of Reagent 1 and mixing. Following the addition of Reagent 2, the red coloration intensified and gradually changed to a reddish-purple color. The development of a reddish-purple color is attributed to the formation of a violet-colored complex between Rhodamine B and antimony salts that are soluble in organic solvents. This color change occurs because Rhodamine B reacts with antimony pentachloride under acidic conditions to form a violet-colored complex that is soluble in the organic phase. The positive identification of Rhodamine B was indicated by a reddish-violet color change, which resulted from the formation of a violet-colored complex between Rhodamine B and antimony salts dissolved in an organic solvent. This reaction occurs when Rhodamine B interacts with antimony pentachloride ($SbCl_5$) in an acidic medium, producing a stable violet complex that is readily soluble in organic solvents [7]. The reaction may be represented by the following simplified equation:

$$\text{Rhodamine B} + SbCl_5 \rightarrow \text{Rhodamine B–}SbCl_5\text{ Complex}$$

In this study, the rapid test kit was employed as a preliminary screening method to detect the presence of Rhodamine B in lipstick samples. This method was selected because it is rapid, simple to perform, and does not require sophisticated laboratory instrumentation. Furthermore, the rapid test kit provides a qualitative indication of Rhodamine B through a characteristic color change following reagent addition. Therefore, the method is suitable for identifying samples suspected of containing Rhodamine B prior to further analysis using more specific instrumental techniques.

Based on the rapid test kit analysis of the 17 lipstick samples, five samples tested positive for Rhodamine B, as indicated by the appearance of a reddish-purple coloration. Samples showing positive results were subsequently selected for further analysis using UV–Visible spectrophotometry. In the present study, five samples were subjected to quantitative analysis because they exhibited positive indications of Rhodamine B during the rapid test screening. This approach was adopted to improve analytical efficiency and to focus quantitative measurements on samples with the highest likelihood of containing Rhodamine B. The results obtained from the analysis of the 17 lipstick samples are presented in Table 1. The positive results from the samples were shown in Figure 1.

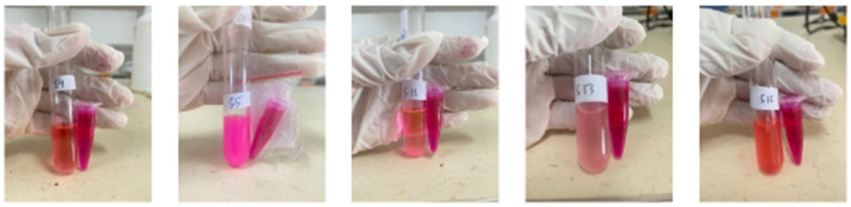

Figure 1. Samples Testing Positive for Rhodamine B

Table 1. Qualitative Analysis of Rhodamine B Using Rapid Test Kit

No	Sample	Color Changed	Test Result
1.	Lipstick 1	Orange	Negative
2.	Lipstick 2	White	Negative
3.	Lipstick 3	Orange	Negative
4.	Lipstick 4	Purple	Positive
5.	Lipstick 5	Purple	Positive
6.	Lipstick 6	White	Negative
7.	Lipstick 7	White	Negative
8.	Lipstick 8	White	Negative
9.	Lipstick 9	Orange	Negative
10.	Lipstick 10	Orange	Negative
11.	Lipstick 11	Purple	Positive
12.	Lipstick 12	Orange	Negative
13.	Lipstick 13	Purple	Positive
14.	Lipstick 14	White	Negative
15.	Lipstick 15	Purple	Positive
16.	Lipstick 16	Orange	Negative
17.	Lipstick 17	Orange	Negative

A positive result was characterized by the development of a purple coloration in the sample, whereas negative samples did not exhibit this characteristic color change and instead produced other colors, including orange, pink, or white. The differences in color responses among the tested samples may reflect variations in the types and compositions of colorants used in the lipstick formulations. These qualitative screening results suggest that lipstick products suspected of containing Rhodamine B remain available in the marketplace, particularly among products that lack official regulatory authorization and adequate ingredient information on their packaging.

3.2 Quantitative Analysis of Rhodamine B

In this study, quantitative analysis was performed using UV–Visible spectrophotometry to determine the concentration of Rhodamine B in lipstick samples. The samples selected for quantitative analysis were those previously identified as potentially positive for Rhodamine B based on the rapid test kit's qualitative screening results. A total of five samples were suspected of containing Rhodamine B during the qualitative analysis and were therefore subjected to subsequent quantitative determination.

Prior to quantitative analysis, the maximum absorption wavelength (λmax) of the Rhodamine B standard solution was determined to ensure that absorbance measurements were conducted under optimal analytical conditions. Determination of λmax is an essential step in UV–Visible spectrophotometric analysis, as measurements at λmax provide the highest sensitivity and accuracy for quantitative analysis. The maximum absorption wavelength (λmax) is the wavelength at which a compound absorbs light most strongly due to electronic excitation, resulting in the highest absorbance. Measurements conducted at λmax are preferred because this wavelength provides the greatest sensitivity to concentration changes, allowing small variations in analyte concentration to produce measurable differences in absorbance. Consequently, quantitative analysis performed at λmax yields more sensitive and reliable analytical results [8]. Wavelength-scanning analysis revealed that the maximum absorption wavelength (λmax) of Rhodamine B was 552.4 nm. This finding is in close agreement with previous studies reported that Rhodamine B exhibits characteristic absorption within the wavelength range of 400–800 nm, while identified a λmax value of 552 nm for a Rhodamine B standard solution. The similarity between the present

result and those reported in the literature confirms the suitability of the analytical conditions employed in this study for the quantitative determination of Rhodamine B [9-10]. The wavelength scanning spectrum and the determination of λmax are shown in Figure 2.

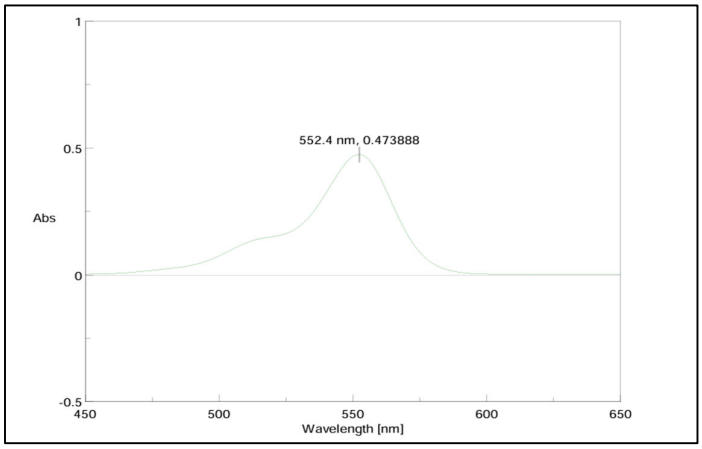

Figure 2. Maximum Wavelength of Rhodamine B

Linearity is a parameter used to evaluate the ability of an analytical instrument to produce a response that is directly proportional to the concentration of an analyte within a specified range. Linearity testing is performed by analyzing a series of standard solutions at different concentration levels. The relationship between analyte concentration (x-axis) and instrument response (y-axis) is plotted to construct a calibration curve. This relationship is expected to be linear and can be expressed by the simple linear regression equation, $y = a + bx$, where y represents the instrument response and x represents the analyte concentration. In this equation, a is the intercept, which indicates the point at which the regression line intersects the y-axis, while b is the slope, which reflects the rate of change in instrument response with respect to concentration. The linearity of the calibration curve is evaluated using the coefficient of determination (R^2). An R^2 value of ≥ 0.999, or a value approaching 1, indicates excellent linearity and demonstrates that the analytical method can produce reliable quantitative results over the tested concentration range [11]. The linear regression analysis performed using the concentrations of Rhodamine B standard solutions and their corresponding absorbance values yielded the regression equation $y = 0.1046x + 0.1053$, with a coefficient of determination (R^2) of 0.9992 (Figure 3.). The high R^2 value indicates an excellent linear relationship between concentration and absorbance over the studied concentration range. An R^2 value approaching 1 demonstrates that the analytical response is directly proportional to the analyte concentration, confirming that the calibration curve exhibits satisfactory linearity. Therefore, the linearity criterion was considered acceptable, indicating that the method is suitable for the quantitative determination of Rhodamine B.

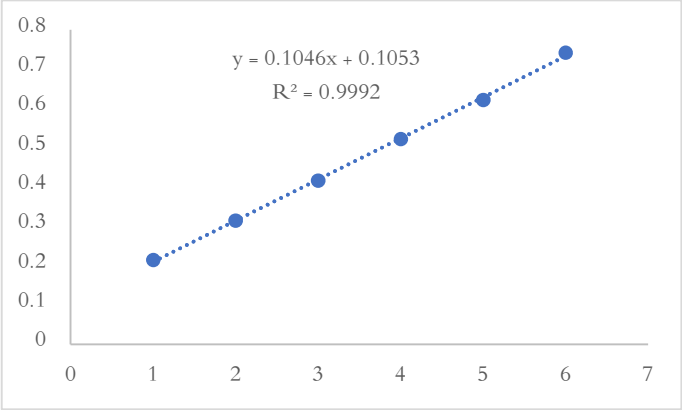

Figure 3. Linearity Curve of Rhodamine Standard

Five lipsticks that were qualitatively positive prepared. This preparation aimed to extract Rhodamine B from the lipstick matrix, remove potential interfering substances, and obtain a clear and homogeneous solution suitable for accurate analysis using UV–Visible spectrophotometry. In this study, 1 g of lipstick sample was used for each analysis. The sample was subsequently treated with 4 N hydrochloric acid (HCl) to create an acidic environment that could enhance the extraction efficiency of Rhodamine B from the sample matrix. Acidic conditions facilitate the release of Rhodamine B from its interactions with other formulation components, thereby promoting its transfer into the organic solvent and improving extraction efficiency [12]. Methanol was selected as the extraction solvent because Rhodamine B exhibits good solubility in this polar organic solvent. In addition, methanol has minimal absorbance in the visible wavelength region and therefore does not interfere with the absorbance measurement of the target analyte during UV–Visible spectrophotometric analysis [13]. The homogenization process was performed to ensure the uniform extraction and distribution of Rhodamine B within the solvent.

The resulting extract was subsequently filtered to obtain a clear filtrate. This filtration step was intended to remove suspended solid particles that could cause light scattering and adversely affect the accuracy of absorbance measurements obtained by UV–Visible spectrophotometry. The filtrate was then transferred into a 25 mL volumetric flask and diluted to volume with methanol. This dilution step was carried out to ensure that the analyte concentration fell within the linear range of the UV–Visible spectrophotometric method, thereby allowing the measured absorbance values to be accurately compared with those of the Rhodamine B standard calibration curve [14]. The result of quantitative analysis of Rhodamine B in samples was shown at Table 2.

Table 2. Determination of Rhodamine B Concentration in Lipstick Samples

Sample	Absorbance	Sample Concentration (ppm)	Sample Concentration (mg/g)	Average Sample Concentration (mg/g)	Deviation Standard
Sample 4	0,6022	4,7504	0,1187	0,1213	0,0023
	0,6171	4,8929	0,1223		
	0,6196	4,9168	0,1229		
Sample 5	0,4623	3,4130	0,0853	0,0861	0,0011
	0,4641	3,4302	0,0857		
	0,4710	3,4961	0,0874		
Sample 11	0,3501	2,3403	0,0585	0,0584	0,0001
	0,3497	2,3365	0,0584		
	0,3500	2,3393	0,0584		
Sample 13	0,5043	3,8145	0,0953	0,0961	0,0008
	0,5096	3,8652	0,0966		
	0,5098	3,8671	0,0966		
Sample 15	0,5563	4,3116	0,1077	0,1082	0,0005
	0,5596	4,3432	0,1085		
	0,5594	4,3413	0,1085		

The results of the quantitative determination of Rhodamine B in lipstick samples indicate that the use of this prohibited synthetic dye is still present in cosmetic products available on the market. Rhodamine B is a synthetic colorant whose use in cosmetic formulations is prohibited due to its toxic and potentially carcinogenic properties when exposure occurs over an extended period. The prohibition of Rhodamine B in cosmetic products is formally regulated under the Regulation of

the Indonesian Food and Drug Authority (BPOM RI) No. 23 of 2019 concerning Technical Requirements for Cosmetic Ingredients, which lists Rhodamine B among substances prohibited for use in cosmetic formulations.

The variation in Rhodamine B concentrations among the analysed samples suggests differences in formulation practices and the extent of unauthorised colourant addition [14]. Sample 4 exhibited the highest Rhodamine B concentration, reaching 0.1213 mg/g, indicating the relatively extensive use of this dye to enhance lipstick color intensity. The presence of Rhodamine B in lipstick products poses a significant health concern because these cosmetics are applied directly to the lips and may be unintentionally ingested during use. Continuous exposure to Rhodamine B has been associated with adverse health effects, including skin irritation, liver dysfunction, and an increased risk of carcinogenicity, as reported in previous toxicological studies [13].

The findings of the present study are consistent with those of several previous investigations that reported the presence of Rhodamine B in lipstick products marketed in traditional markets, particularly among products lacking official marketing authorization. These findings highlight the continuing non-compliance of certain manufacturers with cosmetic regulations and emphasize the need for stricter monitoring and enforcement to ensure the safety of cosmetic products distributed in the marketplace.

5. CONCLUSION

Qualitative analysis using a Rhodamine B rapid test kit indicated that five of the seventeen lipstick samples were positive for Rhodamine B. Quantitative determination by UV–Visible spectrophotometry at 552.4 nm confirmed the presence of Rhodamine B in the selected samples. The calibration curve showed excellent linearity ($R^2 = 0.9992$), demonstrating the suitability of the method for quantitative analysis. The highest Rhodamine B concentration was found in Sample 4 (0.1213 mg/g). The results indicate that prohibited synthetic dyes are still present in some lipstick products marketed in Wonosobo, highlighting the need for stricter regulatory monitoring of cosmetic products available to consumers.

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CONFLICT OF INTEREST: The author declares no conflict of interest.

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