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Analysis of Rhodamine B on Lip Tint Products Containing Natural Red Color Extracts Using UV-VIS Spectrophotometry

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ABSTRAK

Rhodamin B digunakan untuk pewarna tekstil, cat, kertas, atau pakaian. Rhodamin B bersifat karsinogenik dan dapat memicu pertumbuhan sel kanker jika digunakan dalam jangka panjang, serta dapat mengiritasi saluran pernapasan, mata, kulit, dan saluran pencernaan. Penelitian ini bertujuan untuk mengetahui kandungan Rhodamin B dalam lip tint ekstrak alami berwarna merah. Kandungan Rhodamin B ditentukan dengan menggunakan kromatografi lapis tipis dan metode spektrofotometri UV-Vis untuk mengetahui kadar Rhodamin B. Hasil identifikasi menunjukkan hasil positif dengan kadar Rhodamin B pada sampel B sebesar 8,32 ± 0,18 mg/L, sampel C sebesar 17,63 ± 0,35 mg/L, dan sampel D sebesar 8,99 ± 0,13 mg/L. Berdasarkan hasil penelitian ini, masih ditemukan penggunaan Rhodamin B sebagai zat pewarna dalam lip tint yang diklaim berbahan dasar ekstrak alami, dan melanggar ketentuan BPOM yang melarang bahan tambahan Rhodamin B dalam kosmetik.

Kata Kunci: Rhodamin B, Lip tint, Bahan Alami, Kromatografi Lapis Tipis, Spektrofotometri UV-Vis.

ABSTRACT

Rhodamine B is used for textile dyes, paint, paper, or clothing. Rhodamine B is carcinogenic and can trigger the growth of cancer cells if used in the long term, irritating the respiratory tract, eyes, skin, and digestive tract. This study aims to determine the content of Rhodamine B in red natural extract lip tint. Rhodamine B content was determined by thin-layer chromatography and the UV-Vis spectrophotometry method for determining Rhodamine B levels. The identification results showed positive results with Rhodamine B levels in sample B of 8.32 ± 0.18 mg/L, sample C of 17.63 ± 0.35 mg/L, and sample D of 8.99 ± 0.13 mg/L. Based on the results of this study, there is still the use of Rhodamine B as a coloring agent in lip tint that has claims from natural extracts, even though this is prohibited from being used as an additional ingredient in cosmetics by BPOM.

Keyword: *Rhodamine B, Lip tint, Natural Material, Thin Layer Chromatography, UV-Vis Spectrophotometry*

1. Introduction

Lip tint is a lip cosmetic formulated to provide natural-looking color while maintaining lip hydration. This product has the property of absorbing into the lips and producing a color appearance similar to the original color of the lips. Lip tint has the advantage of being enriched with vitamin E, vitamin C, moisturizing agents, and glyceride compounds that help keep the lips hydrated [1]. One of the ingredients used in lip tint formulations is a coloring agent. Synthetic dyes are more popular and often used by cosmetic manufacturers because they are economical and offer stronger, more consistent, and stable coloring power [2]. However, the use of synthetic dyes can also have adverse health effects [3]. Some lip tint products use natural ingredients to add additional benefits. Several studies have researched lip tint formulations with natural ingredient extracts

as coloring agents, such as beetroot extract (*Beta vulgaris* L.) [1], sappan wood (*Caesalpinia sappan* L) [4], kepok banana flower (*Musa paradisiaca* L.) [5], and red dragon fruit extract to produce natural dyes [6].

In addition to being biodegradable and environmentally friendly, plant-based dyes are also non-allergenic and non-toxic compared to synthetic dyes [2]. Due to their harmful effects, some synthetic dyes are banned in various countries. With the growing interest in natural ingredients as coloring agents in lip tint products, consumer health safety is becoming increasingly important. The synthetic dye that is usually used to give a bright red color is Rhodamine B. The Indonesian government, through the BPOM Regulation No. 16 of 2024 regulates the limits of contaminants in cosmetics, including dyes and preservatives, has identified more than 30 hazardous dyes, including Rhodamine B [7]. This ban is based on the cumulative toxic effects of this chemical, which can negatively impact human health.

Long-term use of Rhodamine B in food can lead to liver dysfunction or cancer, while short-term exposure to large amounts can cause acute poisoning [8]. Rhodamine B can irritate the gastric mucosa, eyes, skin, and respiratory tract, and may also trigger the development of liver cancer [9]. However, research indicates that Rhodamine B is still commonly found in many cosmetics, such as in lip tint [10,11], blush on, lipstick, and eyeshadow [7,12–14]. The BPOM identified 16 cosmetic products from 5 companies containing Pigment Red 53, Rhodamine B, and Sudan III. BPOM reported cases of contamination with Pigment Red 53, Rhodamine B, and Sudan III continuing to occur from October 2021 to August 2022 [15].

The identification of Rhodamine B content in lip tint can be performed using Thin Layer Chromatography (TLC) and UV-Vis Spectrophotometry. Thin Layer Chromatography is a qualitative method that detects a sample by separating its components based on polarity [10]. UV-Vis Spectrophotometry is a quantitative method that offers a high level of accuracy [16].

This study aims to determine the content of Rhodamine B in red natural extract lip tint especially those circulating through e-commerce platforms with the best-selling category. Therefore, research on natural extract lip tint is needed to increase public awareness of the potential for safer and higher quality cosmetic ingredients for consumers. In addition, it is expected to provide information so that consumers avoid purchasing products containing rhodamine B.

2. Research Methods

This study will use an experimental method to determine the levels of Rhodamine B in lip tint with bright red natural extracts. This approach aims to ensure the claim of natural extract content in the lip tint used in the experimental procedure.

2.1. Materials dan Intrumentation

The samples used are 5 Liptint products circulating on e-commerce Shopee and Tokopedia with the best-selling category, and the lip tint used is bright red. The samples used contain natural ingredient extracts. Sample A contains strawberry extract, sample B contains watermelon extract, sample C contains Plum extract, sample D contains cranberry extract, and Sample E contains Cherry extract. The materials used in this study included propanol (Merck), ammonia (Merck), ethanol (Merck), Rhodamine B dye (Merck), hydrochloric acid (Merck), distilled water. The instrumentation used in this research is UV-Vis spectrophotometry (Shimadzu UV1780), thin layer chromatography (TLC) plate (Merck), and ultraviolet light lamp chamber.

2.2. Procedure

a. Sample Preparation

In the preparation of thin-layer chromatography test samples, a 500 mg lip tint sample was weighed, and four drops of 4 M HCl were added, followed by 5 mL of ethanol. Ethanol was then added until the total volume reached 10 mL. The mixture was filtered using filter paper [17]. In sample preparation with UV-Vis spectrophotometry, 2 grams of lip tint were weighed, and 16 drops of 4 M HCl solution were added. The

mixture dissolved with 30 mL of ethanol. After the stirring process was complete, the mixture was filtered using filter paper. At this stage, about 2-3 mL of the first filtrate should be discarded, and then the filtering process was carried out once again until the lip tint melt became clear and clear. The clear filtrate was then collected in a 50 mL measuring flask. The concentration was sufficient by adding ethanol until it reached the specified limit mark [18].

b. Qualitative Analysis of Rhodamine B with Thin Layer Chromatography

The mobile phase was prepared by mixing propanol and 25% ammonia in a 90:10 ratio. This mobile phase solution was put into the chamber and closed, then incubated until the eluent reached saturation. The filter paper put into the chamber had been completely wetted beforehand. Rhodamine B Standard Solution is made by using 50 mg of Rhodamine B dye in a 100 mL measuring flask and then adding ethanol to the limit mark [17]. In the qualitative analysis, a 10 x 10 cm TLC plate was activated by heating in an oven at 100°C for 30 minutes. A spotting point, or lower boundary line, was marked on the plate at 1 cm from the bottom edge and 1.5 cm from the top edge, with a 1.5 cm distance between spots. The sample solution and test solution were applied to the TLC plate using a capillary pipette. The plate was left for a moment to allow the solution to absorb and distribute evenly across the plate before drying. The spotted TLC plate was then placed into a chamber containing a saturated mobile phase, removed, and allowed to dry at room temperature. Once dry, the TLC plate was examined under UV light at a wavelength of 254 nm. Visually, the presence of Rhodamine B dye in positive samples appeared as pink spots, with pink or orange fluorescence under ultraviolet light [18]. After observing the spots on the TLC plate, the next step is to calculate the Rf value of the sample (A, B, C, D, and E) and compare it with the Rf value of the comparator (Rhodamine B) following **equation (1)** [10,19].

$$\mathrm{Rf} = \frac{\text{Distance traveled by the compound}}{\text{Distance traveled by the solvent front}} \quad \ldots (1)$$

c. Determination Rhodamine B Content with UV-Vis Spectrophotometric

A total of 5 ml of rhodamine-B solution was measured for maximum absorbance at a wavelength of 400-700 nm using a 96% ethanol blank. The standard series solutions with concentrations of 1, 2, 3, 4, and 5 ppm and samples were measured for absorbance at a maximum wavelength of 545 nm using a UV-Vis spectrophotometer. The concentration and absorbance of the standard were made into a calibration curve, and the absorbance of the sample was entered into the linear regression equation obtained [9]. The Samples identified as containing rhodamine B were measured using a UV-Vis spectrophotometer at maximum wavelength. The treatment was repeated three times, and the total rhodamine B content was calculated.

3. Result and Discussion

3.1. Identification of Rhodamine B

Qualitative analysis of the lip tint samples was carried out using the Thin Layer Chromatography (TLC) method and obtained qualitative results in **Table 1**. These results indicate that samples B, C, and D tested positive for Rhodamine B. The parameters used in the TLC method are in the form of retention factor (Rf) values. TLC is carried out by using a mobile phase and a stationary phase. The mobile phase is prepared by mixing propanol and 25% ammonia in a ratio of 90:10 [17]. Using this solvent mixture because it provides good separation.

This mobile phase is non-polar, while the stationary phase is silica gel GF 254, which is polar so that the standard solution and sample can be separated due to differences in polarity. The separation method in TLC is based on polarity, these compounds are separated due to differences in polarity and affinity of the analyte to the stationary phase and mobile phase [11]. This mobile phase solution is inserted into the chamber and closed, then incubated until the eluent reaches saturation. Eluent saturation is carried out to ensure that the mobile phase particles are evenly distributed throughout the chamber. This aims to ensure that the process of spot movement above the stationary phase by the mobile phase takes place optimally and avoids the tailing process on the TLC plate so that the results are more symmetrical [20].

Table 1. Results of Rhodamine B analysis by Thin Layer Chromatography and UV-Vis Spectrophotometric

Sample	Rf Value	Result	Rhodamine B Content (mg/L)
A	0.83	(-)	-
B	0.93	(+)	8.32 ± 0.18
C	0.93	(+)	17.63 ± 0.35
D	0.91	(+)	8.99 ± 0.13
E	0.96	(-)	-

The identification of TLC samples was carried out by activating a 10 x 10 cm TLC plate, heated in an oven at 110°C for 30 minutes. This activation process removes residual washing solvents and activates the plate's silanol and siloxane groups [21]. A spotting point or lower boundary line was marked on the plate, positioned 1 cm from the bottom edge to prevent submersion by the eluent, and 1.5 cm from the top edge to mark the maximum distance traveled by the eluent. Spotting distance must be carefully considered to avoid spot spreading; using too much sample can decrease resolution and cause spot widening, which may interfere with accurate Rf values [22].

The solution is left briefly to allow it to be absorbed and evenly distributed on the drying plate. The TLC plate, now spotted, is placed in the chamber filled with a saturated mobile phase, then removed and dried at room temperature. Saturating the eluent ensures that mobile phase particles are evenly distributed throughout the chamber, optimizing the movement of spots along the stationary phase and minimizing tailing on the TLC plate [20]. For the dried TLC plate, the next step is to examine it under UV light with a wavelength of 254 nm. Visually, it looks pink, and under ultraviolet light, it shows pink or orange fluorescence. This indicates the presence of Rhodamine B dye in positive samples [18].

Based on the results can be seen in **Table 1**, the Rf value obtained in the sample and comparison solution, the Standard Rf value is 0.93 cm, the Rf value in each sample in sample A is 0.83 cm, in sample B which is 0.93 cm, sample C which is 0.93 cm, sample D which is 0.91 and sample E which is 0.96. There are four samples containing Rhodamine B (samples B, C, and D) because the Rf value of the sample ≥ standard Rf. The Rf value is said to be positive if the sample and the standard have the same Rf value or have a difference in Rf value ≤ 0.2 [22]. The difference between the Rf value of the sample and the standard solution is the same or close to each other with a price difference.

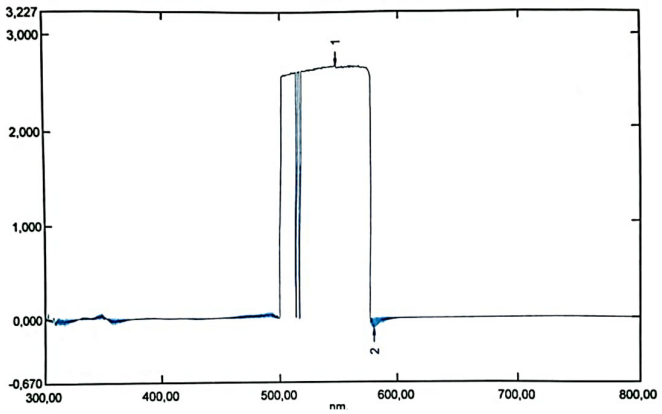

Figure 1. Maximum wavelength curve of Rhodamine B

3.2. Rhodamine B Content in Natural Extract Lip Tint

In determining the content of rhodamine B in lip tint containing natural ingredient extracts, the maximum absorbance was obtained at a wavelength of 547 nm with an absorbance of 2.671 (**Figure 1**). This aligns with research, which found that the maximum wavelength of Rhodamine B is around 545 nm [23]. Variations in maximum wavelength can occur due to differences in equipment conditions and the preparation of standard solutions, which can impact result accuracy. The measurement results of the standard solution obtained a calibration curve with a linear equation for Rhodamine B is $y = 0.0382x + 2.5$ with $R^2 = 0.8295$, which shows

a good correlation coefficient (**Figure 2**). The R^2 value is considered good if it is > 0.5, as R^2 ranges from 0 to 1. The closer the R^2 value is to 1, the better the model's fit [24].

Based on the results in **Table 1**, the determination of Rhodamine B levels in lip tints containing natural ingredient extracts revealed varying amounts of this substance. The analysis confirmed positive results for Rhodamine B, with levels measured at 8.32 ± 0.18 mg/L in sample B (watermelon extract), 17.63 ± 0.35 mg/L in sample C (plum extract), and 8.99 ± 0.13 mg/L in sample D (cranberry extract). The highest concentration was found in sample C with plum extract, it has a darker red color than the other lip tint samples. As supported by qualitative data from the TLC test results, which showed a fluorescent pink color similar to that of the standard solution.

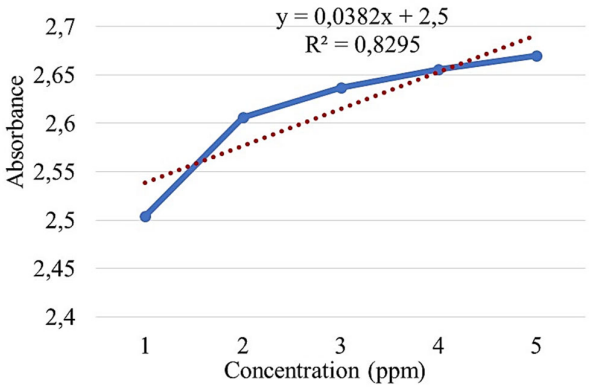

Figure 2. Linear regression of the Rhodamine B curve

These findings indicate that Rhodamine B is still being used as a colorant in lip tints, even those marketed as containing natural ingredients. The results obtained, 3 out of 5 samples have violated the BPOM Regulation No. 16 of 2024 regulates the limits of contaminants in cosmetics, including dyes and preservatives, which prohibits the addition of harmful dyes into cosmetics [7]. Rhodamine B is prohibited for use in lipstick under national and international cosmetic regulations, even in trace amounts. It is sometimes added to lipstick to create vibrant, long-lasting colors, and its relatively low cost makes it appealing to manufacturers [22]. However, its use poses health risks. High levels of Rhodamine B can be dangerous, as increased concentrations lead to greater toxic effects. Frequent exposure to Rhodamine B can cause it to accumulate in the body, potentially resulting in skin and respiratory tract irritation, liver dysfunction, or even liver cancer [18].

4. Conclusions

In conclusion, Rhodamine B is still being used as a colorant in lip tints, including those marketed as containing natural ingredients, despite its prohibition by BPOM as a cosmetic additive. The analysis confirmed the presence of Rhodamine B at levels of 8.32 ± 0.18 mg/L in sample B (watermelon extract lip tint), 17.63 ± 0.35 mg/L in sample C (plum extract lip tint), and 8.99 ± 0.13 mg/L in sample D (cranberry extract lip tint). These findings highlight the need to raise public awareness about choosing safer, higher-quality cosmetic products. Further research is recommended to develop reliable, natural, and safe alternatives to synthetic dyes in lip tints

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