

Original Article

Analysis of Rhodamine B in Lip Creams from Marketplace

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Abstract: Lip cream is a cosmetic product with a creamy texture that provides long-lasting color for the lips. One of the cosmetic colorants often misused as a lip colorant is Rhodamine B, which is supposed to be used for textile coloring. Rhodamine B may cause irritation, is carcinogenic, and can even cause liver damage in high concentrations. This study aims to determine whether lip creams sold in marketplaces contain Rhodamine B and measure the concentration of Rhodamine B in these products. Qualitative analysis of the Rhodamine B content in lip cream was carried out using the thin layer chromatography (TLC) method with the stationary phase silica gel GF254 and the mobile phase n-butanol: ethyl acetate: 25% ammonia with a ratio of 10:4:5. Of the 5 samples that were qualitatively analyzed using TLC, 3 samples were positive for containing rhodamine B, samples number 1, 2, and 3. Quantitative analysis was conducted to determine Rhodamine B concentration using the spectrophotometry UV-Vis method with a maximum wavelength of 546 nm. The results of assay determination showed that the average concentration containing rhodamine B was 0.044% in sample number 1, 0.060% in sample number 2, and 0.052% in sample number 3.

Keywords: Lip cream; Rhodamine B; Spectrophotometry UV-Vis; Marketplace

1. INTRODUCTION

The development of cosmetics has undergone significant transformation over time, reflecting changes in trends, technology, and consumer needs [1]. The majority of cosmetic purchases through online stores are because the prices are cheaper. There are many cosmetic products circulating on the market and online, one of which is lip cream. Along with the times and increasing lifestyles, the use of cosmetics has increased nowadays, especially for lip coloring. Cosmetic products such as lip cream have become very popular because they can beautify the appearance of the lips with various colors and textures [2]. Lip cream is a cosmetic product for the lips that has a creamy texture and usually provides a matte or satin finish. Lip cream is known for its high pigmentation, so it can provide intense color [3]. However, along with increasing demand, there is also the risk of dangerous ingredients in cosmetic products, especially through online platforms [4].

Currently, there are many misuses of synthetic colors, especially for cosmetics. Synthetic colors are usually used by cosmetic manufacturers because they are stronger, more affordable, and more stable, but they can have bad effects on health [5]. Rhodamine B is a synthetic dye whose use in cosmetics is prohibited and is declared a dangerous substance according to the Regulation of Indonesian Food and Drug Authority Number HK.03.1.23.08.11.07517 of 2011 concerning Technical Requirements for Cosmetic Ingredients because it can cause liver, kidney and lymph damage followed by anatomical changes in the form of organ enlargement [6]. Rhodamine B in high

concentrations can cause liver damage. Even though it has been banned by the government, the use of dangerous synthetic dyes is still not widely used, especially for cosmetic dyes. The distribution of lip cream through marketplaces adds to the challenges in monitoring the quality of cosmetic products. Cosmetic products often do not go through adequate due diligence and product safety. This raises concerns about the existence of lip cream products containing Rhodamine B circulating in marketplaces

Based on this description, researchers are interested in conducting research with the title analysis of rhodamine B in lip cream circulating via marketplaces using spectrophotometry UV-Vis. This research is important to ensure the safety of cosmetic products on the market, protect consumers from health risks posed by dangerous substances, and provide information that can be used by authorities to improve regulation and supervision of cosmetic products. Samples obtained from marketplaces are selected with the cheapest and best-selling prices in the shop and do not have well-known brands or registration numbers.

2. MATERIALS AND METHOD

2.1. Materials

Tools used in this research include analytical scales (Ohaus), volume pipettes (Pyrex Iwaki), tweezers, water baths, metal spatula, and spectrophotometry UV-Vis (Shimadzu). The materials used in this study were lip cream samples sold in marketplaces with criteria no brand and have no BPOM license., lipstick samples, 2% ammonia, 10% ammonia, 25% ammonia pro analysis, silica gel GF254, ethanol 70%, ethanol 96% (pro analysis), Rhodamine B, ethyl acetate, n-butanol, acetic acid 10%.

2.2. Quantitative Analysis of Rhodamine B

Qualitative analysis was carried out using the thin-layer chromatography (TLC) method. The stationary phase of silica gel GF 254 is activated by heating at a temperature of 105°C for 5 minutes. The mobile phase in the identification of n-butanol-ethyl acetate - ammonia 25%) with a ratio of 10:4:5. The results were visually observed, the stain appeared pink, and under UV light 254 nm and 366 nm, it fluoresced or orange. Then the R_f value is calculated, and the result is declared positive if the spots between the sample and the standard are the same or close to each other with a difference of ≤ 0.2 [7].

2.3. Qualitative Analysis of Rhodamine B

The first stage carried out in determining the maximum wavelength. Pipet 3 ml of 100 ppm rhodamine B solution (parts per million) using a volume pipette and put it into a 100 ml volumetric flask (concentration 3 ppm), then add 96% ethanol to the mark and homogenize. The maximum absorption was measured at a wavelength of 400-800 nm using a blank. The blank used is 96% ethanol [8]. Next, determine the calibration curve by making a standard solution of rhodamine B with a concentration of 7,9,11,13,15 ppm for each solution. Then the absorbance is measured at the maximum wavelength obtained and 96% ethanol is used as a blank to obtain a calibration vs absorbance curve.

Sample preparation with 1 gram of sample put into an Erlenmeyer and then soaked in 10 ml of 2% ammonia solution dissolved in 70% ethanol with a ratio of 1:2. The solution is filtered and then heated over a water bath. The residue from evaporation was dissolved in 10 ml of 10% acetic acid solution. Next, the wool thread is put into the solution and heated for 10 minutes. Then the wool yarn is washed with distilled water and placed in a 10% ammonia base solution. The solution is heated until the color of the wool thread dissolves, then the alkaline solution obtained is used as a sample in

the spectrophotometric UV-Vis reading. The test solution was extracted, put into a cuvette then put into a spectrophotometer and measured at the maximum wavelength obtained, and as a blank 96% ethanol was used [9].

2.4. Data Analysis

Quantitative analysis was used to calculate rhodamine B levels using UV-Vis spectrophotometry, then statistical analysis was carried out by determining linear regression. From the UV-Vis spectrophotometry method data in the form of absorbance data which is calculated using the equation $y = bx + a$. Calculation of Rhodamine B levels in the preparation sample using the formula below:

$$C = \frac{X \times V \times F}{w}$$

Information

C = Rhodamine B content in the sample (mg/L)

X = Rhodamine content after dilution (mg/L)

V = Sample volume (ml)

F = Dilution Factor

W = Sample weight (mg)

3. RESULTS AND DISCUSSION

Sampling was carried out on lip cream samples that were red in color, cheap in price, and did not have number registration with the Indonesian Food and Drug Authority (BPOM), totaling 5 samples with various brands and shops sold through the marketplace. Organoleptic tests use the five senses based on shape, color, smell, and texture. The organoleptic test results can be seen in Table 1.

Table 1. Organoleptic test results

Parameter test	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Shape	Cream	Thick liquid	Cream	Cream	Thick liquid
Smell	fragrant	Fragrant	Fragrant	Not smell	fragrant
Texture	Smooth and slightly oily	Smooth and lumpy	Smooth, color does not fade easily	Smooth and slightly oily	a little sticky

The characteristics of lip dyes that contain rhodamine B are that the color is bright and more striking, sometimes the color does not look homogeneous, there is a strong odor, there are lumps of color on the product, it does not include a code, label, brand, content information or other complementary identity [5]. Rhodamine B can be detected by a color change that is easily observed visually. A pink color change occurs when Rhodamine B is dissolved in an aqueous or ethanol solution, and the more concentrated the color, the higher the concentration of Rhodamine B [10].

3.1. Qualitative Analysis of Rhodamine B

The method used is the thin layer chromatography method which is a technique for separating compounds using the principles of adsorption and partition coefficient [11]. The TLC method was chosen because it is affordable, specific in separating one component from other components, and

simple to work with [12]. The principle of TLC is to separate samples based on differences in polarity known as the "like dissolve like" principle, meaning that polar likes polar and non-polar likes non-polar. The results of the TLC analysis can be seen in Figure 1 and Table 2.

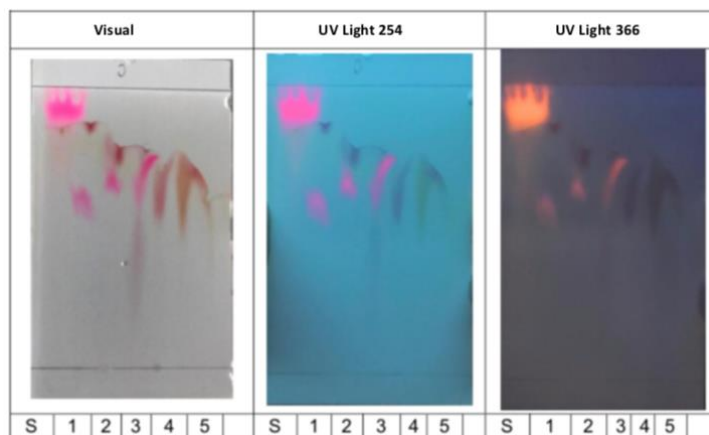


Figure 1. TLC Profile. (S) Standart of Rodhamin B (1) sample 1 (2) Sample 2 (3) Sample 3 (4) Sample 4 (5) Sample 5

Table 2. The results of the TLC analysis

Sample	Spotting			RF	Result
	Visual	UV 254	UV 366		
Rhodamine B Standard	Pink	Fluorescent	orange	0.93	Positive
Sample 1	Pink	Fluorescent	Orange	0.73	Positive
Sample 2	Pink	Fluorescent	Orange	0.75	Positive
Sample 3	Pink	Fluorescent	Orange	0.87	Positive
Sample 4	Purple	Does not fluoresce	Purple	0.74	Negative
Sample 5	Purple	Does not fluoresce	Purple	0.87	Negative

Rhodamine B exhibits a bright red color, which intensifies under UV light. Based on research if the stain containing the rhodamine B compound is visually seen it is pink, when viewed under UV light with a wavelength of 366 nm it will fluoresce yellow or orange [13].

3.2. Quantitative Analysis

The quantitative test using UV-Vis spectrophotometry aims to determine the levels of Rhodamine B contained in lip cream circulating via marketplaces. This spectrophotometric method has a principle, namely the Lambert-Beer law, where Lambert-Beer law states that the concentration of a substance is directly proportional to the amount of light absorbed, or inversely proportional to the logarithm of the light transmitted [11].

Substances that can be analyzed using spectrophotometry UV-Vis are substances in solution form and substances that appear colored because Rhodamine B is a compound or molecule that gives color due to the presence of a chromophore group, where the chromophore group is quinine. The quantity of color produced by Rhodamine B is very sharp. This is caused by the presence of an autochrome group, where the autochrome group is dimethyl amine. So a quantitative examination of the sample was carried out using UV-Vis spectrophotometry [14]. In quantitative analysis, the maximum wavelength in the rhodamine B solution is determined first. The results of determining

the maximum wavelength with a wave range of 400-800 nm obtained a wavelength of 546 nm. The results of this research are almost similar to previous research, namely that a maximum wavelength of 545 nm was obtained [8]. The purpose of making a standard curve is to determine the linearity of the concentration of the rhodamine B standard solution with the absorbance value [15]. From the absorbance results of the standard solution of rhodamine B, a curve was then created which is the relationship between absorbance (y) and concentration (x) to determine the linear equation and relationship coefficient values. The linearity of the rhodamine B solution calibration curve can be seen in Figure 2.

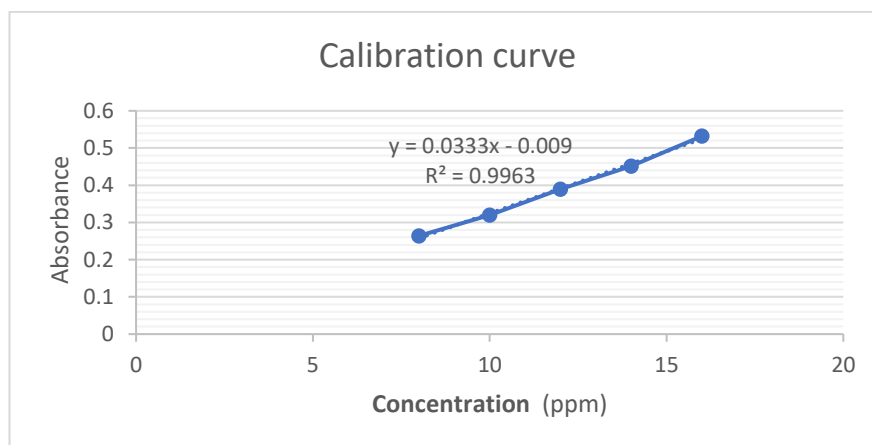


Figure 2. Linearity of the rhodamine B solution calibration curve

The calibration curve shown shows the relationship between environmental concentration (ppm) and absorption. Since the R^2 value is close to 1, it can be concluded that the data has very good linearity; that is, the relationship between concentration and absorption is almost completely linear.

Sample preparation was carried out using the wool yarn absorption method. The principle of the wool yarn absorption method is to draw the dye from the sample into the wool yarn in an acidic atmosphere with heating followed by dissolving it with a base.[14]. The binding mechanism for Rhodamine B in the wool yarn is because the wool yarn is composed of peptide bonds which contain cystine, glutaric acid, aspartic acid lysine, and arginine bonds. Rhodamine B can pass through the cuticle layer by converting the sestine into cysteine by an acid. Cysteine is formed by breaking down the SS bonds of cystine in an acidic environment. The opening of these bonds causes the entry of Rhodhamin B into the wool thread. Rhodamine B enters the wool yarn and binds to COO^- from aspartic acid and also binds to $+\text{NH}_3$ from Arginine [11]. The binding mechanism of rhodamine B in wool yarn can be seen in Figure 3.

The wool thread will release the dye, and the dye will enter the alkaline solution. This solution will be a sample sample for quantitative analysis using spectrophotometry UV- Vis [16]. In the TLC results, 3 of the 5 lip cream samples contained rhodamine B, so the samples containing rhodamine B were continued with quantitative analysis using the Uv-Vis spectrophotometric method. From the results, it is known the percentage of rhodamine B found in lip cream-type cosmetics, where 3 out of 5 lipstick samples do not meet cosmetic requirements because there is the synthetic dye Rhodamine B. The concentration of rhodamine B levels can be seen in Table 3.

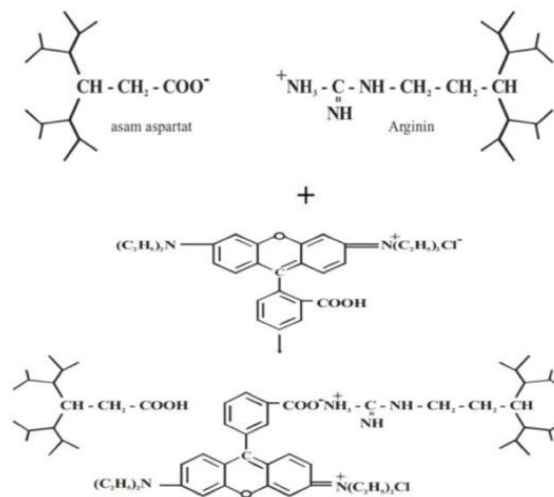


Figure 3. Mechanism of rhodamine B in wool

Table 3. Results of Rodhamine B concentration in lip cream samples

Samples	Replication	Concentration (%)	Average Concentration	SD
1	1	0.044	0.044%	0.00047
	2	0.045		
	3	0.044		
2	1	0.057	0.060%	0.00245
	2	0.063		
	3	0.060		
3	1	0.052	0.052%	0.00082
	2	0.054		
	3	0.053		

Based on Indonesian Food and Drug Authority regulation No. 23 of 2019 concerning technical cosmetic ingredients, cosmetics distributed must meet the requirements, namely, cosmetics must use ingredients that meet standards, and be registered and obtain a distribution permit from Indonesian Food and Drug Authority. Rhodamine B dye is prohibited from being added to the manufacture of cosmetics according to the Regulation of the Head of the Indonesian Food and Drug Authority regulation No. 18 of 2015 concerning Technical Requirements for Cosmetic Ingredients because it can cause negative effects on health. Rhodamine should not be used in cosmetics, especially as a dye. Rhodamine B can cause acute effects if ingested at 500mg/kg BW, which is a toxic dose. The possible toxic effect is gastrointestinal irritation [6].

Many studies and investigations have found that lip cream or lipstick products sold online, especially inexpensive ones that are not registered with Indonesian Food and Drug Authority, carry a higher risk of containing Rhodamine B. Additionally, many cosmetics such as face creams sold in marketplaces contain hazardous ingredients like mercury and hydroquinone [12]. Therefore, Indonesian Food and Drug Authority plays a crucial role in supervising the safety of cosmetics, especially those sold online.

4. CONCLUSION

Based on the research results of Rhodamine B analysis on lip cream samples sold online, it was found that 3 out of 5 samples were identified as positive for containing rhodamine B, in samples number 1, 2, and 3. The results of quantitative analysis, using UV-Vis spectrophotometry, obtained

average levels. The average content containing rhodamine B was 0.044% in sample number 1, 0.060% in sample number 2, and 0.052% in sample number 3.

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Conflicts of interest: The authors reported no potential conflicts of interest.

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