

Analysis Of Rhodamine B Dye Content In Lipstick At Beriman Tomohon Market

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ABSTRAK

Penggunaan kosmetik seperti lipstik bertujuan untuk memperbaiki penampilan alami bibir dan menimbulkan perubahan warna. Masalah yang sering dikhawatirkan saat ini adanya zat pewarna terlarang yang terkandung pada komponen utama sediaan lipstik. Rhodamin B adalah pewarna sintetik yang penggunaannya dilarang dalam kosmetika khususnya lipstik. Penelitian ini bertujuan untuk menganalisis zat pewarna Rhodamin B pada Lipstik yang tidak memiliki izin edar dari BPOM yang beredar di pasar Beriman Tomohon. Metode penelitian secara Kromatografi Lapis Tipis (KLT) yang dilanjutkan dengan pengukuran panjang gelombang pada Spektrofotometri UV-Vis. Populasi penelitian adalah seluruh sediaan lipstik yang bewarna merah dan sebagai sampel diambil 10 (sepuluh) jenis lipstik berbeda. Analisis data pada penelitian ini adalah mencari nilai RF yang diperoleh dari KLT kemudian membuat grafik dari persamaan $y = bx + a$ lalu mencari nilai x untuk mengukur kandungan Rhodamin B pada lipstik.. Hasil penelitian ini didapatkan 1 (satu) sampel lipstik label L(I) positif mengandung Rhodamin B. Secara visual lipstik label L(I) memiliki warna merah muda dan berfluoresensi orange saat disinari UV 366nm, Rhodamin B memiliki nilai Rf 0.85 sedangkan sampel L(I) memiliki nilai Rf 0.84. Rhodamin B memiliki panjang gelombang 544nm dan sampel lipstik L(I) memiliki absorbansi 0.362 dengan kadar Rhodamin B sebesar 0.4115%.

Kata kunci : Rhodamin B, Lipstik, KLT, Spektrofotometri UV-Vis

ABSTRACT

The use of cosmetics such as lipstick aims to improve the natural appearance of the lips and induce discoloration. A problem that is often worrying about today is the presence of prohibited dyes in lipstick preparations' main components. Rhodamine B is a synthetic dye prohibited in cosmetics, especially lipsticks. This research aims to analyze the Rhodamine B dye in lipsticks that do not have a distribution permit from BPOM circulating in the Beriman Tomohon market. The research method was Thin Layer Chromatography (TLC), followed by wavelength measurement on UV-Vis Spectrophotometry. The research population was all red lipstick preparations and 10 (ten) different lipsticks as a sample. Data analysis in this research is to find the RF value obtained from TLC then make a graph of the equation $y = bx + a$ then look for the x value to measure the Rhodamine B content in lipstick. The results of this research were obtained from 1 (one) sample of lipstick label L(I) positive containing Rhodamine B. Visually, the lipstick of the L(I) label has a pink color and fluorescence orange when irradiated with 366nm UV, Rhodamine B has an Rf value of 0.85. In contrast, the L(I) sample has an Rf value of 0.84. Rhodamine B has a wavelength of 544nm, and the lipstick sample L(I) has an absorbance of 0.362 which has a Rhodamine B content of 0.4115%.

Keywords : Rhodamine B, lipstick, TLC, UV-Vis Spectrophotometry

1. INTRODUCTION

An attractive appearance can create high attractiveness, making a person more confident. One of them is the use of makeup or cosmetics on women. Blushing cheeks, lustrous and charming eyelashes, and lips that are bright red and not pale make women more enthusiastic about doing activities inside and outside the home.

The definition of cosmetics is a material or preparation used on the external parts of the human body such as the epidermis, hair, nails, lips, and external genital organs, or teeth and oral mucous membranes, especially for cleaning, perfuming, changing appearance or improving body odor and keeping the body in good condition¹. Cosmetics are not used to treat or prevent disease, so they are not drugs. Cosmetic preparations include foundation, loose powder, face cream, and lipstick.

Lipsticks are designed to improve the natural appearance of the lips, cause discoloration, enhance shine, and smooth wrinkle lines and creases².

Oils, waxes, fats, and dyes are the main components of lipstick preparations³. Unfortunately, many irresponsible people still mix lipstick's main components with banned coloring substances to get the color many women like. One of the forbidden coloring substances is Rhodamine B.

Rhodamine B is a synthetic dye that colors paper, textiles, and ink. Rhodamine B is a carcinogenic substance and, if used repeatedly, can irritate the respiratory tract, irritation to the skin, irritation to the eyes, irritation to digestion, poisoning, impaired liver function, and liver cancer⁴. Rhodamine B has been banned in Europe since 1984, while in Indonesia, according to the Menteri Kesehatan regulation in 1996, Rhodamine B is one of the dyes prohibited from being used as a cosmetic additive because it can harm the body⁵.

In October 2022, Badan Pengawas Obat dan Makanan Republik Indonesia found 16 products containing banned and hazardous substances, including Red 3 and Red K10, also known as Rhodamine B⁶. The findings led BPOM to cancel distribution licenses, recall orders, and secure products from circulation.

Research conducted at Lirung market by using qualitative methods, showed that two (two) lipstick samples were positive for Rhodamine B. This is said to be positive

because the lipstick samples have the same visual color as Rhodamine B, which is pink after being viewed under UV light, and the sample fluorescence is orange, just like Rhodamine B⁷.

Based on the case description above, the author is interested in analyzing the presence or absence of Rhodamine B dye content in lipstick circulating in Beriman Tomohon Market.

2. RESEARCH METHOD

Tools and Materials

The tools used in this research are capillary pipes, porcelain cups, chambers, analytical scales, volume pipettes, measuring pipettes, spatulas, stirring rods, filter paper, funnels, ointment pots, beaker glass, UV-Vis spectrophotometry.

The materials used in this research included 10 (ten) different types of lipstick, Rhodamine B, Klt 60 GF254 plate, hydrochloric acid (HCl), 95% methanol (CH3OH), ammonia, n-Butanol (C4H9OH), ethyl acetate, and anhydrous sodium sulfate (Na2SO4).

Population and Samples

This research's population consisted of all red-colored lipsticks sold by cosmetic traders at Beriman Tomohon Market. The samples included 10 (ten) types of red lipstick that did not have a distribution permit from BPOM and were sold by 5 (five) different traders (2 kiosks and 3 street vendors).

Place and Time Research

This research was conducted at the Laboratory of Pharmaceutical Chemistry and Pharmaceutical Biology FMIPA Universitas Kristen Indonesia Tomohon from January to March 2024

Research Type and Research Design

This type of research method is experimental research in the laboratory using Thin Layer Chromatography (TLC) to compare and analyze the presence of Rhodamine B content in lipstick sold by traders in Beriman Tomohon Market. Furthermore, wavelength measurements and absorbance values of Rhodamine B and lipstick sample test solutions were carried out, and Rhodamine B levels were calculated in lipstick samples using UV-Vis spectrophotometry.

Qualitative Analysis of Rhodamine B

1. Preparation of Test Solution

Preparation of Test Solution labeled with L(A), L(B), L(C), L(D), L(E), L(F), L(G), L(H), L(I), L(J). 2 g of sample was put into a porcelain cup. Then, 16 drops of 4 M Hydrochloric Acid and 30 mL of methanol were added and melted in a water bath. Then, it is filtered with filter paper filled with Anhydrous Sodium Sulfate. The filtrate is taken and concentrated again in a water bath; the concentrated solution is put in a 50 mL volumetric flask and then added with methanol until the limit mark line and homogenized⁸.

2. Preparation of Comparator Standard Solution

Preparation of Rhodamine B comparative standard solution labeled with (RB) as a positive control. 50 mg of Rhodamine B was dissolved with 10 mL of methanol and shaken until dissolved. As a negative control, 5 mL of pure methanol solution was used.

3. Identification of Sample by Thin Layer Chromatography

Identification of samples with TLC is as follows⁹:

a. The 10 x 7 cm TLC plate was activated by heating in an oven at 105°C for 30 minutes.

b. Solutions of L(A), L(B), L(C), L(D), L(E), L(F), L(G), L(H), L(I), L(J), and RB were photographed on the TLC plate using a capillary tube with a distance between stains of 0.6 cm, upper limit distance of 2 cm, lower limit distance of 1 cm, and a substance distance of 7 cm, then left for a while to dry.

c. The TLC plate containing the stain is inserted into the chamber, filled with the mobile phase of n-Butanol, Ethyl Acetate, and Ammonia (55:20:25).

d. The phase moves up until it is almost near the upper limit of the TLC plate. Then, the TLC plate is lifted and allowed to dry in free air.

e. Visually observe the stain under ultraviolet light; if visually the stain is pink and under ultraviolet light is orange, it indicate the presence of Rhodamine B content.

f. Calculation of the Rf value

The Rf value can be calculated after the spots on the TLC plate are marked.

The Rf value can be defined as follows¹⁰:

$$Rf = \frac{\text{Distance traveled by stain}}{\text{Distance traveled by eluent}}$$

This identification was carried out 3 (three) times to minimize the possibility of errors in the research and build confidence in the data obtained.

4. Quantitative Analysis of Rhodamine B

Comparison Standard Solution. 0.1 g of Rhodamine B was dissolved with 30 mL of methanol in a 100 mL volumetric flask. Methanol was added until the limit mark line was reached, then homogenized to obtain a solution of Rhodamine B 1000 ppm. Furthermore, 10 mL of 1000 ppm Rhodamine B solution was pipetted and put into a 100 mL volumetric flask. Then, methanol was added until the limit mark line, and a standard 100 ppm Rhodamine B was obtained¹¹

Determination of Maximum Wavelength of Rhodamine B Solution. Rhodamine B standard solution was diluted to 38 ppm by pipetting 19 mL of 100 ppm Rhodamine B solution and put into a 50 mL volumetric flask, added methanol until the limit mark line, and homogenized. Then, the maximum absorption was measured at a wavelength of 400-800 nm using a blank. The blank used is methanol.

Calibration Curve Creation. A standard solution calibration curve was prepared using 100 ppm Rhodamine B solution. The Rhodamine B solution was pipetted as much as 15 mL, 19 mL, 23 mL, 27 mL, and 31 mL was put into a 100 mL volumetric flask then each volume was added to methanol until the 50 mL limit mark and homogenized, so that a concentration series of 30 ppm, 38 ppm, 46 ppm, 54 ppm, and 62 ppm were obtained. The concentration is then measured at the maximum wavelength obtained previously using methanol as a blank, and a calibration curve will be obtained.

Sample Content Determination. A total of 1 mL of test solution filtrate in a 50 mL volumetric flask was pipetted and then put into a 25mL volumetric flask. Merhanol was added until the limit line was homogenized. The absorbance was measured at the maximum wavelength that had been

determined. This measurement was carried out three times¹².

Data Analysis

Determination of the comparison standard calibration curve using the formula $y = bx + a$ using the Microsoft Excel application¹³. Rhodamine B content in lipstick samples can be summed in the formula¹⁴ :

$$K = \frac{X \cdot V}{B \cdot s}$$

Description :

K = Rhodamine B content in the sample (mg/g)
X = Concentration of Rhodamine B (mg/mL)
V = Sample Volume (mL)
Bs = Sample Weight (g)

3. RESULTS AND DISCUSSION

Thin Layer Chromatography (TLC)

Qualitative identification begins with activating the 60 GF254 TLC plate in the oven

at 105°C for 30 minutes. Then, 10 (ten) types of lipstick sample test solution and Rhodamine B comparison standard solution were bottled on a 10x7 cm TLC plate, the TLC plate was inserted into the chamber which already contained a mobile phase namely n-butanol, ethyl acetate, and ammonia (55:0:25).

Ethyl acetate and ammonia have polar properties, while n-butanol is semipolar. This mobile phase is used to separate the stains between the sample components and Rhodamine B well. Furthermore, the mobile phase can move up to the upper limit line on the TLC plate.

The TLC plate is lifted then air dried and observed by the naked eye, forming the compound's separation point and bringing UV light at 366nm. UV light at a wavelength of 366nm stains will fluorescence with various colors while the plate will be dark¹⁵. The results of sample identification with TLC can be seen in the figure.

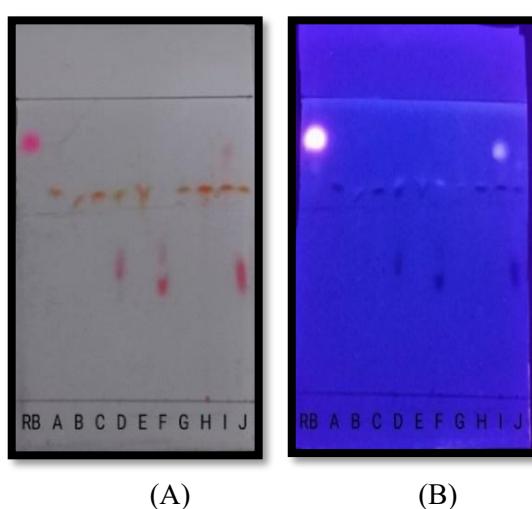


Figure 1. (A) TLC result by the naked eye, (B) after 366nm uv irradiated.

Figure A shows that the RB sample has a pink color; samples L(A), L(B), L(C) have an orange color; sample L(D) has 2 (two) consecutive stains with pink and orange colors; sample L(E) has an orange color; sample L(F) stain 1 is pink and stain 2 is faint pink; sample L(G) is orange; sample L(H) is orange; sample L(I) stain 1 is orange, stains 2 and 3 are faint pink; sample L(J) stain 1 is pink and stain 2 is orange. Figure B, when the plate is illuminated with UV 366nm, sample RB fluorescence

orange, samples L(A), L(B), L(C), L(D), L(E), L(F), L(G), L(H), and L(J) turn into brown color while sample L(I) fluorescence orange just like Rhodamine B.

Compounds containing Rhodamine B will be easily observed because they are visually pink and, when viewed under UV light, will give a fluorescence orange color¹⁶. The following R_f values are obtained from TLC identification.

Table 1. Rf Value Of RB Solution and Lipstick Sample Test Solution

No.	Sample	Repetitions	Rf Value
(1)	(2)	(3)	(4)
1	RB	I	0.85
		II	0.85
		III	0.87
2	L(A)	I	0.68
		II	0.7
		III	0.72
3	L(B)	I	0.67
		II	0.68
		III	0.72
4	L(C)	I	0.67
		II	0.7
		III	0.72
5	L(D)	I	0.48; 0.65; 0.68
		II	0.48; 0.68
		III	0.51; 0.57
6	L(E)	I	0.67
		II	0.7
		III	0.7
7	L(F)	I	0.48; 0.58
		II	0.35; 0.52; 0.7
		III	0.48; 0.57
8	L(G)	I	0.67
		II	0.7
		III	0.68
9	L(H)	I	0.68
		II	0.7
		III	0.7
10	L(I)	I	0.7; 0.82; 0.87
		II	0.7; 0.78; 0.84
		III	0.71; 0.74; 0.85
11	L(J)	I	0.47; 0.7
		II	0.45; 0.7; 0.78
		III	0.51; 0.77; 0.79

Description : + = Positive ; - = Negative

The results of the Rf calculation between 10 (ten) types of lipstick sample test solutions and Rhodamine B comparator standard solution. Rhodamine B has an Rf value of 0.85, and Sample L (I) has an Rf value of 0.84.

After qualitative analysis using the TLC method, it was found that 1 (one) sample was suspected positive for Rhodamine B, namely the sample labeled L(I). To confirm TLC's positive results, quantitative analysis was continued by measuring the wavelength of Rhodamine B and the absorbance of lipstick sample L(I).

UV-Vis Spectrophotometry

Measure the maximum wavelength by taking 19 mL of standard solution of Rhodamine B 100 ppm and then put into a 50 mL volumetric flask and adding methanol until the limit mark line. A Rhodamine B standard solution concentration of 38 ppm was obtained. Measured the maximum absorption at wavelengths between 400-800 nm using a blank. The blank used is methanol¹⁷. The blank serves to correct the reading or spectrum of the sample.

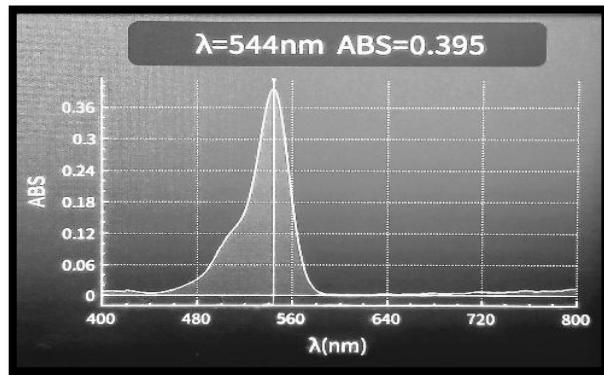
**Figure 2.** The wavelength of Rhodamine B

Figure 2. Shows the results of measuring the wavelength of Rhodamine B at a concentration of 38 ppm which is 544nm with an absorbance of 0.395. Furthermore, the wavelength of 544nm will be used to measure the wavelength of the concentration series of Rhodamine B and to measure the absorbance of lipstick samples.

Measurement of absorbance of the standard curve was pipetted Rhodamine B

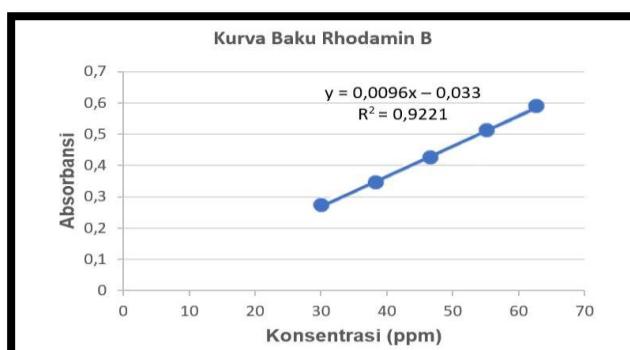
solution as much as 15 mL, 19 mL, 23 mL, 27 mL, and 31 mL put into a measuring flask of 100 mL then each volume was added to methanol until the limit mark of 50 mL and homogenized. Furthermore, a concentration series of 30 ppm, 38 ppm, 46 ppm, 54 ppm, and 62 ppm was obtained. The concentration was then measured at a wavelength of 544nm using methanol as a blank.

Table 2. Absorbance result of Rhodamine B Standard Curve

Concentration (ppm)	Absorbance
(1)	(2)
30	0.288
38	0.308
46	0.398
54	0.446
62	0.603

Table 2 shows the results of Rhodamine B absorbance measurements from concentrations of 30 ppm; 38 ppm; 46 ppm; 54 ppm; 62 ppm. According to the Lambert-Beer law, a good absorbance range is ($0.2 \leq A < 0.8$) because the reading error

rate by UV-Vis spectrophotometry is minimal in that range¹⁸. After the absorbance of each concentration has been found, the next step is to make a standard curve between absorbance (y) and concentration (x) to determine the linear equation.

**Figure 3.** Calibration Curve Of Rhodamine B Standard Solution

The results of determining the calibration curve of the standard solution of Rhodamine B with concentrations of 30 ppm, 38 ppm, 46 ppm, 54 ppm, and 62 ppm measured at a wavelength of 544nm obtained the standard curve equation in figure 3 that $y = 0.0096x - 0.033$ with a coefficient of determination R^2 of 0.9221. So, it can be said that there is a positive correlation between levels and absorbance. This means that the absorbance will also increase when the concentration is increased.

In the coefficient of determination test, a good R^2 value is more than 50% because it means that the sample used for regression can represent at least half of the total population and can explain more relevant¹⁹.

Absorbance measurements of 10 (ten) types of lipstick samples at a wavelength of 544nm were carried out with as many as 3 (three) repetitions on each sample.

Table 3. Absorbance Of Lipstick Sample

Sample	Absorbance I	Absorbance II	Absorbance III
(1)	(2)	(3)	(4)
L(A)	0.055	0.054	0.054
L(B)	0.121	0.123	0.116
L(C)	0.137	0.137	0.134
L(D)	0.106	0.106	0.100
L(E)	0.190	0.190	0.188
L(F)	0.095	0.094	0.089
L(G)	0.174	0.177	0.177
L(H)	0.096	0.104	0.102
L(I)	0.365	0.362	0.362
L(J)	0.195	0.195	0.189

Table 3. shows that the L(I) sample has an absorbance with the applicable vulnerability according to the Lambert-Beer law, namely ($0.2 \leq A < 0.8$). This absorbance value shows the alignment between the results of qualitative analysis, namely the TLC method, and quantitative analysis, which measures the wavelength and absorbance using UV-Vis spectrophotometry. Calculate the amount of Rhodamine B substance in lipstick samples by entering the absorbance value in the equation $y = 0.0096x - 0.033$. It was found that the level of Rhodamine B compound in L(I) lipstick was $41.15 \mu\text{g/mL}$ with a percentage level of 0.4115%.

Rhodamine B on the lips will irritate the lips with wounds and even swelling, which can interfere with the appearance that is not expected for every woman in cosmetics²⁰. The use of lipstick on the lips allows the lipstick to be swallowed with saliva or food and drink consumed, so if the lipstick used contains Rhodamine B, it can have a negative impact on health directly or indirectly because Rhodamine B has toxicity, carcinogenic mutagenic, and irritating properties for health.

4. CONCLUSION

The results of qualitative analysis of Rhodamine B and 10 (ten) types of lipstick samples using TLC obtained 1 (one) positive sample containing Rhodamine B, namely sample L (I). Seen with the naked eye, Rhodamine B is pink, and sample L (I) is also pink. When illuminated UV 366nm, Rhodamine B and sample L(I) fluorescence orange. Rhodamine B has an R_f value of 0.85, while the L(I) sample has an R_f value of 0.84.

The maximum wavelength measurement of Rhodamine B is 544nm. The absorbance of sample L(I) was 0.362. The level of Rhodamine B compound in sample L(I) is $41.15 \mu\text{g/mL}$ with a percentage content of 0.4115%.

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