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# ANALYSIS OF RHODAMINE B IN LIPSTICK SOLD ON MARKETPLACE X USING TLC METHOD (THIN LAYER CHROMATOGRAPHY)

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**Abstract**

**Introduction:** Rhodamine B is a chemical dye used for textile dyes and is prohibited by BPOM in cosmetics, especially lipsticks. This study aims to determine the presence of Rhodamine B contained in lipstick preparations sold in Marketplace X.

**Method:** A total of 13 lipstick samples sold on Marketplace X were selected based on predetermined criteria. This study used silica gel $GF_{254}$ stationary phase with several compositions and types of mobile phase, namely ethyl acetate:methanol:ammonia (15:6:3), ethyl acetate:methanol:ammonia (25:6:1), n-butanol:ethanol absolute:aquades (20:12:15), ethyl acetate:methanol:(ammonia+aquades) (15:3:3), ethyl acetate:methanol:ammonia (15:3:3), and n-butanol:ethanol absolute:aquades (55:20:25). The mobile phase was selected based on the criteria of $R_f$ value of 0.2-0.8 and good separation with a value of Rs 1.5. The data were processed and presented in tabular form, and analyzed using descriptive analysis to determine the description of Rhodamine B.

**Results:** The optimal mobile phase with n-butanol ethanol absolute:aquadest (55:20:25) resulted in an average Rf value of 0.76 and an Rs value of 3.96. The results showed 2 form 13 samples were positive Rhodamine B (code L3 and L7) with a difference of $R_f$ 0.2 with orange fluorescence stains. Consumers are expected to be vigilant in choosing cosmetic products by looking at the distribution permit number.

**Conclusion:** This study concludes that the optimal mobile phase for separating Rhodamine B were n-butanol ethanol absolute:aquadest (55:20:25). A total of 2 positive samples (code L3 and L7) from 13 lipstick samples.

***Keywords***: *Rhodamine B, Lipstick, Thin Layer Chromatography, Optimization, Mobile Phase*

## INTRODUCTION

The more increase for cosmetic purposes, cosmetics with various prices and brands will appear. A marketplace s a platform that markets electronic products by bringing together many buyers and sellers to transact with each other (Apriadi et al., 2017). With the development of today's era, women have switched to using advanced technology for everything. One of them is how to buy goods through the Marketplace. According to the Central Statistics Agency in 202,0, as many as 25.72% of sales accounts on Marketplace, and in 2019 sales of cosmetics sold on E-commerce increased by 8.87%, based on a survey in January 2018 it was found that the majority of online shopping consumers were women with a figure reached 65% (BPS, 2021). The use of cosmetics increases every year, data according to BPOM shows that the number of registered cosmetic products from 2017-2021 is 212,980 products. There was an increase in 2021 starting from July - December by 60.01% as many as 82,165 products (BPOM, 2021).

Rhodamine B is a substance that contains carcinogenic properties and in excess levels can cause liver damage. The use of lipsticks containing Rhodamine B can cause adverse effects such as irritation to inflammation in the lip area which results in decreased appetite. If it occurs in the long term it can cause cancer (Syamsuri, 2017). Evidence found in animal testing of Rhodamine B based on toxicity test has an LD50 2000 mg/kg causing strong irritation of the mucous membranes and toxicity tests with subcutaneous and oral injections to cause local sarcoma carcinogens, while in IV LD50 89.5 mg/kg kidney enlargement occurs, liver and spleen (Ristianingrum et al., 2018). BPOM has banned the use of Rhodamine B with another name red K10 or coloring agent CI 45170 in cosmetic preparations (BPOM RI, 2019).

## METHOD

### Tools and Materials

The tools used include TLC silica gel 60 $F_{254}$ plates (merck), oven (Ika), analytical balance (Ohaus), porcelain cup, measuring cup (Iwaki) 25 mL, volumetric flask (Iwaki) 100 mL, 25 mL, and 10 mL, UV lamp (CAMAG), chamber (CAMAG), water bath, stirring rod, watch glass, funnel (Iwaki), filter paper, and dropper, bulb pipette, spatula, beaker glass (Iwaki) 100 mL, micropippet 20 µL (Soccorex).

The materials used include: Lipstick, Rhodamine B p.a (Sigma-aldrich), methanol absolute pa (Supelco), ethyl acetate p.a (Supelco), ammonia p.a (Supelco), n-Butanol p.a (Supelco), ethanol absolute p.a (Supelco), aquadest, HCl p.a

**Procedures**

1. **Sampling**
   The sample used in this research is lipstick sold on Marketplace X. A total of 13 samples of lipstick sold on Marketplace X were selected based on predetermined sampling criteria.
2. **Mobile Phase Optimization of Samples**
   a. **Mobile Phase Creation**
      The mobile phase used consists of:
      1) n-Butanol : Ethanol absolute : Aquadest (20 : 12 : 15) (Fatimah *et al*., 2016)
      2) Ethyl acetate : Methanol : Ammonia (25 : 6 : 1) (Ratnaningtiyas, 2013)
      3) Ethyl acetate : Methanol : Ammonia (15 : 6 : 3) (Riyanti *et al*., 2018)
      4) Ethyl acetate : Methanol : (Ammonia+Aquadest) (15:3:3) (Jusnita dan Nandu, 2016)
      5) Ethyl acetate : Methanol : Ammonia (15:3:3) (Jusnita dan Nandu, 2016)
      6) n-Butanol : Ethanol absolute : Aquadest (55:20:25) (Riyanti *et al*., 2018)

      The method of making the mobile phase is done by inserting the mobile phase into a 30 mL volumetric flask with the stated ratio (Lero, 2021).
   b. **Preparation of Comparative Standard Solution**
      An amount of 10 mg of standard Rhodamine B dye was dissolved in a 100 mL volumetric flask with methanol pa to the limit mark. The solution was homogenized (100 µg/mL) (Elfasyari *et al*., 2020).
   c. **Preparation of Test Solution**
      A total of 2 grams of the lipstick sample was poured into a porcelain cup, then 16 drops of 4 M concentrated HCl were added and 20 mL of methanol was added and then melted over a water bath until it melted. Furthermore, the liquid is filtered through filter paper that has been given 0.1 g of anhydrous sodium sulfate and the filtrate is taken. Then put in a water bath to be concentrated, then the filtrate is put into a vial. Then 2 ml of the filtrate was taken and 1 mL of Rhodamine B standard solution was added with a ratio of 2:1 and then put into the vial (Tjuana *et al*., 2021).
   d. **Determination of Optimum Conditions**
      The selection of the mobile phase was carried out to obtain chromatography with circular, non-widening, and not tailing spots. Optimization was carried out by spotting the Rhodamine B comparison solution spot and then eluted with various mobile phases, namely n-butanol : ethanol absolute : aquadest (20 : 12 : 15), ethyl acetate : methanol : ammonia (25 : 6 : 1), ethyl acetate : methanol : ammonia (15 : 6 : 3), ethyl acetate : methanol :(ammonia+aquadest) (15:3:3), ethyl acetate : metahnol : ammonia (15:3:3), dan n-butanol : ethyl acetate : ammonia (55:20:25).
      The mobile phase was first saturated in the chamber using filter paper for 10 minutes before the plate was inserted into the chamber. The TLC plate was cut to a size of 4x10 cm and was heated in an oven at 100ºC for 30 minutes. Then, 3 L of the standard solution and the test solution were applied using a micropipette starting from a distance of 1 cm below the plate. Between the stains are spaced 1 cm. Then the plate is dried using a hair dryer. The TLC plate that has been dotted with the analyte is inserted into a chamber that has previously been saturated using the mobile phase. The plate was allowed to stand until optimally eluted, then removed, and then dried using room temperature.
      Observe the color visually with 254 nm and 366 nm UV lamps, if the pink spots are visible and under ultraviolet light, they fluoresce pink or orange, it can be said that the sample contains Rhodamine B. The Rf value of the test solution is calculated and then compared with the Rf value of the solution. parent. Then it was replicated three times. Confirmed by using a spray reaction of concentrated HCl pa 4M. Re-observations were made on 254 nm and 366 nm UV

lamps with HCl spray as a reagent to produce orange fluorescence indicating samples containing Rhodamine B (Tjuana *et al*., 2021).

3. **Qualitative Analysis**
   a. **Preparation of Test Solution**
   A total of 0.5 grams of the lipstick sample was poured into a porcelain cup, then 16 drops of concentrated 4 M HCl & 5 mL of methanol were added and then melted in a water bath until melted. Furthermore, the liquid is filtered through filter paper that has been given anhydrous sodium sulfate and the filtrate is taken. Then it is placed in a water bath to be concentrated, then the filtrate is put into a vial (Tjuana *et al*., 2021).
   b. **Sample Identification**
   The TLC plate was cut to a size of 4x10 cm and was heated in an oven at 100°C for 30 minutes. Then smear the standard solution and the test solution using a micropipette as much as 3 µL starting from a distance of 1 cm below the plate. Between the stains are spaced 1 cm. Then the plate is dried using a hair dryer. The TLC plate that has been stained with the analyte is put into a chamber that has previously been saturated using the selected mobile phase, namely n-butanol : ethanol absolute : aquadest (20 : 12 : 15). The plate was allowed to stand until optimally eluted, then removed and then dried using room temperature. Observe the color visually under UV light of 254 nm and 366 nm, if the spots are pink and under ultraviolet light, they fluoresce pink or orange, it can be said that the sample contains Rhodamine B. The Rf value of the test solution was calculated and then compared with the Rf value of the mother liquor. Then it was replicated three times. It was confirmed by using a spray reaction of concentrated HCl pa 4 M. Re-observation was carried out on UV lamps at 366 254 nm and nm with HCl spray as a reagent, which resulted in orange fluorescence indicating samples containing Rhodamine B (Tjuana *et al*., 2021).

## RESULTS

### A. Mobile Phase Optimization Results

The results of the Rf value and resolution obtained from the optimization of the six types of mobile phase composition are presented in the following table.

Table 1. $R_f$ Value and Resolution of Mobile Phase Composition

| Mobile Phase | Sample | $R_f$ result | | Resolution | Information |
|---|---|---|---|---|---|
| | | $R_f$ Rhodamine B (cm) | Nearest Spot $R_f$ (cm) | | |
| **n-Butanol: Ethanol absolute: Aquades (20:12:15)** | + control (Rhodamine B) | 0.66 | | | |
| | Replication 1 | 0.66 | 0.76 | 5.35 | Homogeneous |

| | | | | | |
|---|---|---|---|---|---|
| | Replication 2 | 0.66 | 0.75 | 3.03 | |
| | 3 replication | 0.66 | 0.77 | 3.53 | |
| **Ethyl acetate : Methanol: Ammonia (25:6:1)** | + control (Rhodamine B) | 0.31 | 0.39 | 2.64 | Homogeneous |
| | Replication 1 | 0.3 | 0.38 | 3.05 | |
| | Replication 2 | 0.3 | 0.38 | 3.05 | |
| | Replication 3 | 0.3 | 0.38 | 2.44 | |
| **Ethyl acetate : Methanol: Ammonia (15:6:3)** | + control (Rhodamine B) | 0.8 | 0.84 | 3.53 | Homogeneous |
| | Replication 1 | 0.79 | 0.84 | 5.35 | |
| | Replication 2 | 0.8 | 0.84 | 3.03 | |
| | 3 replication | 0.81 | 0.85 | 3.53 | |
| **Ethyl acetate: Methanol : (Ammonia+Aquades) (15:3:3)** | + control (Rhodamine B) | TD | TD | TD | Inhomogeneous |
| | Replication 1 | TD | TD | TD | |
| | Replication 2 | TD | TD | TD | |
| | 3 replication | TD | TD | TD | |
| **Ethyl acetate : Methanol: Ammonia (15:3:3)** | + control (Rhodamine B) | TD | TD | TD | Inhomogeneous |
| | Replication 1 | TD | TD | TD | |
| | Replication 2 | TD | TD | TD | |
| | 3 replication | TD | TD | TD | |
| **n-Butanol: Ethyl acetate: Ammonia (55:20:25)** | + control (Rhodamine B) | TD | TD | TD | Inhomogeneous |
| | Replication 1 | TD | TD | TD | |
| | Replication 2 | TD | TD | TD | |
| | 3 replication | TD | TD | TD | |
| **Conclusion** | Of the 6 types of mobile phase mixtures, there are 3 types of homogeneous mobile phase mixtures that are used for the optimization of the mobile phase. | | | | |

Description: TD: Not Detected

Based on the results obtained, the best mobile phase was selected for qualitative analysis of the lipstick sample, namely the mobile phase mixture of n-Butanol : Etanol absolute : Aquades (20:12:15).

**B. Results of Sample Identification with Selected Mobile Phase Mixture of n-Butanol : Ethanol absolute : Aquadest (20:12:15)**

The results of the Rf value and the resolution obtained from the identification of samples with the selected mobile phase mixture of n-Butanol: Absolute ethanol: Aquades (20:12:15) are presented in the following table.

Table 2. $R_f$ Value and Resolution of Identification Results of Lipstick Samples with N-Butanol Mobile Phase: Absolute Ethanol: Aquadest (20:12:15)

| Sample | Repetition | $R_f$ Value | Average $R_f$ | Difference $R_f$ | $R_f$ Difference Terms | Information |
|---|---|---|---|---|---|---|
| Control + Rhodamine B | | 0.72 | | | | |
| Sample 1 | I | 0.83 | 0.83 | 0.11 | 0.2 | Positive |
| | II | 0.83 | | 0.11 | | Positive |
| | III | 0.83 | | 0.11 | | Positive |
| Control + Rhodamine B | | 0.76 | | | | |
| Sample 2 | I | 0.76 | 0.77 | 0 | 0.2 | Positive |
| | II | 0.76 | | 0 | | Positive |
| | III | 0.78 | | 0.02 | | Positive |
| Control + Rhodamine B | | 0.73 | | | | |
| Sample 3 | I | 0.725 | 0.73 | 0.006 | 0.2 | Positive |
| | II | 0.725 | | 0.006 | | Positive |
| | III | 0.725 | | 0.006 | | Positive |
| Control + Rhodamine B | | 0.6875 | | | | |
| Sample 4 | I | 0.7125 | 0.71 | 0.025 | 0.2 | Positive |
| | II | 0.7125 | | 0.025 | | Positive |
| | III | 0.7125 | | 0.025 | | Positive |
| Control + Rhodamine B | | 0.78 | | | | |
| Sample 5 | I | 0.80 | 0.80 | 0.02 | 0.2 | Positive |
| | II | 0.80 | | 0.02 | | Positive |
| | III | 0.80 | | 0.02 | | Positive |
| Control + Rhodamine B | | 0.68 | | | | |
| Sample 6 | I | 0.75 | 0.74 | 0.07 | 0.2 | Positive |
| | II | 0.74 | | 0.06 | | Positive |
| | III | 0.74 | | 0.06 | | Positive |
| Control + Rhodamine B | | 0.64 | | | | |
| Sample 7 | I | 0.62 | 0.62 | 0.02 | 0.2 | Positive |
| | II | 0.62 | | 0.02 | | Positive |
| | III | 0.62 | | 0.02 | | Positive |
| Control + Rhodamine B | | 0.79 | | | | |
| Sample 8 | I | 0.81 | 0.81 | 0.02 | 0.2 | Positive |
| | II | 0.81 | | 0.02 | | Positive |
| | III | 0.81 | | 0.02 | | Positive |
| Control + Rhodamine B | | 0.66 | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Sample 9 | I | 0.68 | 0.68 | 0.02 | 0.2 | Positive |
| | II | 0.68 | | 0.02 | | Positive |
| | III | 0.68 | | 0.02 | | Positive |
| Control + Rhodamine B | | 0.75 | | | | |
| Sample 10 | I | 0.75 | 0.75 | 0 | 0.2 | Positive |
| | II | 0.75 | | 0 | | Positive |
| | III | 0.76 | | 0.01 | | Positive |
| Control + Rhodamine B | | 0.74 | | | | |
| Sample 11 | I | 0.76 | 0.76 | 0.02 | 0.2 | Positive |
| | II | 0.76 | | 0.02 | | Positive |
| | III | 0.76 | | 0.02 | | Positive |
| Control + Rhodamine B | | 0.74 | | | | |
| Sample 12 | I | 0.89 | 0.89 | 0.15 | 0.2 | Positive |
| | II | 0.9 | | 0.16 | | Positive |
| | III | 0.9 | | 0.16 | | Positive |
| Control + Rhodamine B | | 0.75 | | | | |
| Sample 13 | I | 0.76 | 0.76 | 0.01 | 0.2 | Positive |
| | II | 0.76 | | 0.01 | | Positive |
| | III | 0.76 | | 0.01 | | Positive |
| Conclusion | | | A total of 13 lipstick samples were positive based on the difference in Rf between the lipstick samples and the Rhodamine B . standard | | | |

**A. Observation Results of Lipstick Samples with Visible Light, UV Light 254nm and 366nm after Spraying 4M HCl**

The results of the observation of lipstick samples with visible light and under UV light of 254nm and 366nm after being sprayed with 4 M HCl are presented in the following table.

Table 3. Results of Observation of Spots in UV Rays of 254nm and 366nm After Spraying with 4 M HCl

| Sample | Visible Light | UV rays | | Information |
|---|---|---|---|---|
| | | 254nm | 366nm | |
| Sample 1 | Pink | TT | TT | Negative |
| Sample 2 | Pink | TT | TT | Negative |
| Sample 3 | Pink | TT | TT | Negative |
| Sample 4 | Pink | T | T | Positive, Fluorescent Orange |
| Sample 5 | Pink | TT | TT | Negative |
| Sample 6 | Pink | TT | TT | Negative |

| | | | | |
|---|---|---|---|---|
| Sample 7 | Pink | T | T | Positive, Fluorescent Orange |
| Sample 8 | Pink | TT | TT | Negative |
| Sample 9 | Pink | TT | TT | Negative |
| Sample 10 | Pink | TT | TT | Negative |
| Sample 11 | Pink | TT | TT | Negative |
| Sample 12 | Pink | TT | TT | Negative |
| Sample 13 | Pink | TT | TT | Negative |

Conclusion: From the 13 lipstick samples, there were 2 positive lipstick samples containing Rhodamine B which were indicated by orange fluorescence stains.

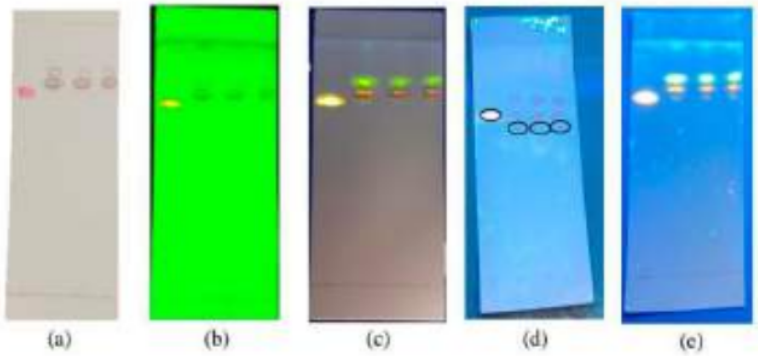


Figure 1. Results of Identification of Positive Stains for Lipstick Samples Code L3 (a) Visible Light (b) 254nm UV Light (c) 366nm UV Light (d) 254 nm UV Light after Spraying (e) 366nm UV Light after Spraying HCl 4 M

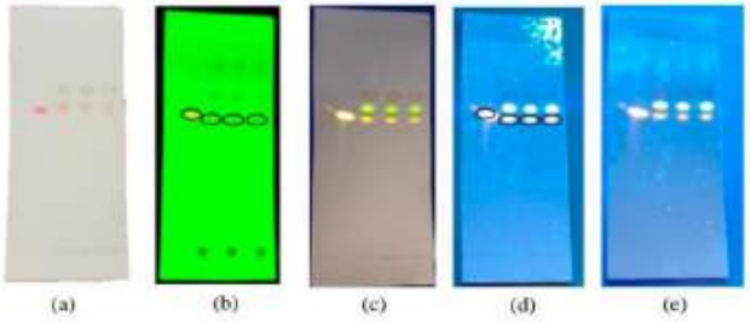


Figure 2. Results of Identification of Positive Stains for Lipstick Samples Code L7 (a) Visible Light (b) 254nm UV Light (c) 366nm UV Light (d) 254 nm UV Light after Spraying (e) 366nm UV Light after Spraying HCl 4 M

**DISCUSSION**

The sample used in this study is lipstick cosmetic preparations with the following criteria: red lipstick, the affordable price is around Rp. 1,000 – Rp. 25,000-star seller shops in the marketplace with a star rate of 4.0 – 4.9 the selection of prices and rates is because many women are interested in buying cosmetics at affordable prices and buying cosmetic products in stores that are widely purchased with good reviews, selected cosmetic packaging in composition using foreign language because if consumers lack understanding in foreign languages so they do not know the hazardous substances contained in the purchased product and the packaging, there is no distribution permit number from the Ministry of Health and a notification number from BPOM because the product has not been registered with BPOM so the product has not been carried out. product safety testing by BPOM.

The separation of Rhodamine B in lipstick samples is influenced by the type and composition of the mobile phase so to obtain an optimal separation, it is necessary to optimize the type and composition of the mobile phase (Ediningtyas, 2012). Optimization of the method is done by optimizing the length of the elution distance and the composition of the mobile phase that has been made (composition I, II, III, IV, V, and VI). A good $R_f$ value is expected in the range of 0.2-0.8 (Gandjar and Rohman, 2012); A good resolution value if you think about a value of 1.5 and produces a round stain without tailings shaped like a ribbon (Wulandari, 2011).

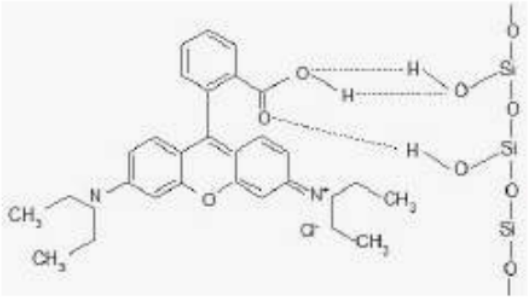

Figure 3. Hydrogen Bonding between Rhodamine and Silanol Group
------- = Hydrogen Bond (Ediningtyas, 2012)

The stationary phase used is silica gel $GF_{254}$ because silica gel has a small and uniform particle size so that resolution and efficient separation can be produced. The surface of the silica gel consists of Si-O-Si groups and Si-OH (silanol) groups. The presence of silanol groups can form hydrogen interactions with the analyte. The hydrogen interaction that occurs between Rhodamine B and the stationary phase is due to the presence of O atoms in Rhodamine B which can form hydrogen interactions with the stationary phase, so Rhodamine B will be retained in the stationary phase (Lero, 2021).

In addition to the analyte, the silanol group can also form hydrogen interactions with aquades which makes the silica gel deactivated so that before use, the silica gel needs to be heated at a temperature of 100°C for 30 minutes. This is done to reactivate the surface of the silica gel so that the silanol groups can interact with the analyte. GF254 shows that the fluorescent compound added to the plate has an excitation wavelength of 254 nm. The compound added is usually in the form of manganese or phosphorus-activated zinc silicate (Ediningtyas, 2012).

The composition of the mobile phase I was based on a previous study conducted by (Fatimah *et al*., 2016) using a mixture ratio of n-butanol: ethanol: aquades (20: 12: 5) with a total volume of 30 mL. Optimization of the composition of the mobile phase needs to be done because the samples used are different. In a study conducted by Fatimah *et al* (2016) the sample studied was a cake containing Rhodamine B. Optimization through the selection of the mobile phase aims to obtain an appropriate composition for the sample used in this study. The sample used in this study is lipstick sold in Marketplace X.

Based on Table 5.3, 6 types of mobile phase mixtures were made, each of which consisted of 3 types of solvents with different polarity indices. The composition of the mobile phase I, namely n-butanol: ethanol: aquades (20: 12: 5), has a polarity index of 6.22; the composition of the mobile phase II, namely ethyl acetate: methanol: ammonia (25:6:1) of 4.70; the composition of the mobile phase III is ethyl

acetate:methanol:ammonia (15:6:3) of 5.28; the composition of mobile phase IV is ethyl acetate:methanol:(ammonia+aquades)(15:3:3) of 5.32; the composition of the mobile phase V, namely ethyl acetate:methanol:ammonia (15:3:3) of 5.31; The composition of mobile phase VI is n-butanol:ethyl acetate:ammonia (55:20:25) of 5.55. The migration speed of the sample components depends on the physicochemical properties of the stationary phase, the mobile phase, and the sample components. The difference in polarity is expected to provide good separation results and can be used for qualitative analysis of the next sample. The mobile phase that has been made is saturated into the chamber. Saturation was carried out using filter paper for 10 minutes before carrying out the elution process. The purpose of saturation of the chamber is to equalize the vapor pressure of the mobile phase used so that the separation can run well (Wulandari, 2011).

The function is given the upper and lower limits of the plate as a marker of the distance traveled by the eluent. The lower limit of the plate is made so that it is not submerged by the eluent. The purpose of spotting is to use a micropipette so that the spotting is small, because in TLC a good spotting is kept as small as possible to avoid widening the stain and if too many samples are used it will reduce the resolution (Yuniarto & Rosalina, 2019). The widening of the stain can interfere with the $R_f$ value because it allows peak crushing to occur. Highlighting is done on the bottom line that has been made. Then let it sit for a while to dry. Next, the plate is carefully inserted into a closed chamber containing the eluent with the mobile phase positioned below the line. This TLC uses the ascending method. After reaching the upper limit,

The composition of the mobile phase I was based on previous research conducted by (Fatimah *et al*., 2016) using a mixture ratio of n-butanol: ethanol: aquades (20: 12: 15) with a polarity index of 6.22. The results of the separation of Rhodamine B in the lipstick sample after being eluted by the mobile phase showed that Rhodamine B could be eluted with an $R_f$ value of 0.67 with symmetrical stains and produced a good resolution value of 1, 2, 3 replications, namely 5.35; 3.03; 3.53 of these results are said to have met the requirements of good resolution because the value obtained is 1.5.

The composition of the mobile phase II is based on a previous study conducted by (Ratnaningtiyas, 2013) using a mobile phase ratio of ethyl acetate: methanol: ammonia (25:6:1) with a polarity index of 4.70. The results of the replication $R_f$ values 1, 2, and 3 were 0.43; 0.42; and 0.42 with the resolution value of replication 1, 2, and 3 of 2.42; 2.53; and 2.55. The results of the separation of Rhodamine B in the lipstick sample after being eluted by the mobile phase showed that Rhodamine B could be eluted with the standard $R_f$ value of Rhodamine B of 0.30625 and the $R_f$ value of replication samples 1, 2, and 3 was 0.3 based on the results obtained that met the requirements. a good range of $R_f$ values is 0.2-0.8. The resolution values obtained from each replication 1, 2, and 3 were 3.05; 3.05; 2, 4375 based on the results obtained, the resolution value has met the requirements, namely 1.5. However, the stains obtained from the separation with the mobile phase did not produce good stains, then the separation between Rhodamine B stains and other stains was too close together.

The composition of the mobile phase III is based on previous research conducted by (Riyanti *et al*., 2018) on lipstick samples, namely by using a ratio of ethyl acetate: methanol: ammonia (15:6:3) with a polarity index of 5.28 resulting from the Rhodamin standard $R_f$ obtained is 0.91. The results of the separation of Rhodamine B in the lipstick sample after being eluted by the mobile phase showed that Rhodamine B could be eluted with the standard $R_f$ value of Rhodamine B of 0.8 and the $R_f$ value of the replication samples 1, 2, 3 was 0.79; 0.8; 0.80 based on the results obtained have met the requirements of a good $R_f$ value range of 0.2-0.8. The resolution value obtained from each replication 1, 2, and 3 was 5.35; 3.03; 3.53 based on the results obtained the resolution value has met the requirements, namely 1.5. However,

The composition of the mobile phase IV-VI is not a good mobile phase because the mixture of the composition is not homogeneous and is formed into 2 separate phases which are divided into an upper and lower layer, the top layer is clear, and the bottom layer is cloudy. This does not meet the requirements of a good eluent, which has sufficient and stable purity (Wulandari, 2011). The solution was separated into 2 phases due to the difference in polarity of each type of solvent, so it was not continued for the separation of Rhodamine B compounds in the lipstick sample because it would affect the separation of the analyte to be not good.

The optimal separation between Rhodamine compounds and other analytes was shown in the

mobile phase of a mixture of n-butanol: ethanol: and distilled water (20: 12: 5). Other analyte stains originating from the mobile phase or other compounds from the lipstick sample did not interfere with the Rhodamine B stain because the Rhodamine B peak was already separated from other nearby stains from the mobile phase or other compounds from the lipstick sample as indicated by the resolution value. which is more than 1.5. The mixed mobile phase of polar and non-polar solvents can separate compounds well because the polar part of the mobile phase will interact with the polar part of the analyte, while the non-polar part will interact with the non-polar part of the analyte (Ediningtyas, 2012).

Next, a qualitative analysis was carried out on the lipstick sample. The addition of HCl destroys the compounds present in the lipstick sample, in this case, the sample is also added with methanol to melt the waxy substance in the lipstick with the help of heating so that the filtrate from the sample can be obtained, also because methanol is a polar organic solvent. and has a low boiling point so that it can dissolve organic substances which are also polar (Yuniarto & Rosalina, 2019). Filtration with anhydrous sodium sulfate aims to reduce the water content of the heating product (Yuniarto & Rosalina, 2019). Heating the TLC plate in an oven with a temperature of 100°C for 30 minutes aims to remove water molecules contained on the TLC plate,

To compare the $R_f$ value and color, a standard comparison solution for the positive control was made. The positive control consisted of Rhodamine B. The eluent used was the eluent that was selected after the previous optimization was n-butanol: absolute ethanol: aquades with a ratio of 20:12:15. The eluent was allowed to saturate for ± 30 minutes for complete separation. The reason for closing the chamber is to ensure that the conditions in the chamber are saturated with solvent vapors (Lero, 2021). To obtain this condition, filter paper is placed in the chamber until it is moistened with solvent. Saturated conditions in the chamber prevent evaporation of the objective solvent from saturation to homogenize the mixture and remove water vapor/other gases in the absorbent phase which will hinder the eluent rate (Yuniarto & Rosalina, 2019).

Then calculate the $R_f$ value, if the identification of the $R_f$ value has the same value then the compound can be said to have the same or similar characteristics. Meanwhile, if the $R_f$ value is different, the compound can be said to be a different compound. The greater the $R_f$ value of the sample, the greater the movement distance of the compound on the thin layer chromatography plate. Then, the addition of a stain-seeking reagent by spraying with 4M HCl is needed to produce colored or fluorescent compound derivatives (Wulandari, 2011).

In general, a combination of aromatic compounds and some unsaturated compounds can absorb UV light (Wulandari, 2011). These compounds can be analyzed using thin layer chromatography with a stationary phase which is then detected by examination under 254 nm UV light. The results of the identification of Rhodamine B are listed in Table 5.4. showed that among the 13 lipstick samples, Rhodamine B was found in sample codes 3 and 7 where the UV light at 254 nm showed a yellow fluorescence sample, and visually observed the stain on the sample code that appeared on the TLC plate was pink. Rhodamine B will give yellow or orange fluorescence when observed at UV light at 254 nm and 366 nm, and pink when viewed visually, in addition, the results are declared positive if the color of the sample spots,

The $R_f$ value of the lipstick sample with sample code 3 (0.71 cm), and sample code 7 (0.62 cm) is almost parallel to the $R_f$ value of the Rhodamine B reference standard solution in sample code 3 of 0.69 cm, and the sample code 7 is 0.74 cm. While in Table 5.15. shows the sample codes 1, 2, 4, 5, 6, 8, 9, 10, 11, 12, and 13 the $R_f$ value shows a difference of 0.2 with the reference standard solution which is parallel to the $R_f$ value of the Rhodamine B reference standard solution, but after sprayed with a spray reagent using 4M HCl the stain does not fluoresce orange when observed under UV light at 254 nm it is said that the stain is not a Rhodamine B stain. The results obtained are the difference between the $R_f$ value of the sample and the reference standard solution 0,

It was concluded that the samples with codes 3 and 7 were positive for Rhodamine B dye and when observed after spraying with 4M HCl then observed under UV light of 254 nm with orange fluorescence. The results of sample codes 1, 2, 4, 5, 6, 8, 9, 10, 11, 12, and 13 were not positive for Rhodamine B, seen from the visual test results that the stains that appeared were not pink and in ultraviolet light. 254 nm and 366 nm did not show the presence of orange fluorescence and the difference between the $R_f$ value of the sample and the reference standard solution was 0.2, the result was declared negative.

## CONCLUSION

Based on the results of the Mobile Phase Optimization research using the TLC (Thin Layer Chromatography) Method on the Analysis of Rhodamine B Content in Lipstick Cosmetic Preparations Sold in Marketplace X, it can be concluded that:

1. The best mobile phase was selected, namely n-butanol: ethanol: aquadest (20:12:15) because the $R_f$ value and resolution obtained had met the requirements, namely for an $R_f$ value of 0.2-0.8 and the best resolution value was 1.5 and resulted in stains that were not widened and not tailing, the separation between Rhodamine B stains was separated from other stains.
2. In the lipstick sample which was analyzed qualitatively using the TLC method with the best selected mobile phase, namely n-butanol: ethanol: aquadest (20:12:15) obtained as many as 2 positive samples from 13 samples, namely samples with codes 3 and 7 where the samples were there is no notification number from BPOM with the difference in the $R_f$ value that is close to the comparison standard $R_f$ value, namely the price difference 0.2. The $R_f$ value of the lipstick sample with the code of sample 3 (0.71) and sample 7 (0.62). That is, the sample has violated the rules that have been made by BPOM which prohibits the addition of harmful ingredients to cosmetics, one of which is Rhodamin B based on BPOM No. 23 of 2019 regarding the technical requirements for cosmetic ingredients, Rhodamine B preparations are substances that are prohibited to be added to cosmetics.

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# ANALYSIS OF RHODAMINE B IN LIPSTICK SOLD ON MARKETPLACE X USING TLC METHOD (THIN LAYER CHROMATOGRAPHY)

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