

ANALYSIS OF RHODAMIN B IN EYESHADOW SOLD ON MARKETPLACE X

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Submission date: 11-Apr-2023 05:11PM (UTC+1000)

Submission ID: 2061374273

File name: ODAMIN_B_IN_EYESHADOW_SOLD_ON_MARKETPLACE_X_USING_TLC_METHOD.pdf (1.59M)

Word count: 4287

Character count: 21423

ANALYSIS OF RHODAMIN B IN EYESHADOW SOLD ON MARKETPLACE X USING TLC METHOD (THIN LAYER CHROMATOGRAPHY)

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Abstract

Introduction: The synthetic dye Rhodamine B is used for coloring paper, textiles, wool, silk, and as a reagent. Rhodamine B is prohibited for food coloring and cosmetics, as it irritates the eyes and skin, poisoning, impairs liver function, and causes cancer. The study aimed to determine the presence of Rhodamine B in eyeshadow sold in the marketplace X.

Method: Identification of Rhodamine B compounds using TLC with mobile phase optimization. The design of this research is experimental with a purposive sampling technique. Sample in pink, red, and deep red, solid powder, imported or local products, no composition label listed, price from Rp. 5,000 – Rp. 50,000, no expired date recorded, and no notification number from National Agency of Drug and Food Control. R_f and Resolution value data are processed using descriptive statistics in the form of tables.

Result: The optimal motion phase is n-butanol : Ethanol : Aquadest (20:12:15), samples that showed positive Rhodamine B produced round/elliptical and tailless stains. Visually, it offers light pink colors, and under UV light, 254 and 366 show yellow or orange. Sample 4 with an average R_f value of 0.64 and Resolution of 3.07. Sample 8 with an average R_f value of 0.66 and Resolution 3.57 and sample 9 with an average of 0.79 and Resolution 2.03.

Conclusion: This study concluded that 3 out of 10 samples contain Rhodamine B.

Keywords: Rhodamine B, Eyeshadow, Thin Layer Chromatography, Mobile Phase Optimization

INTRODUCTION

The pandemic caused people's shopping patterns to change from offline to online by 80%. Data obtained from the Coordinating Ministry for Economic Affairs in 2020 shows an increase in online transactions for body care products, especially cosmetics and spas (Kemenperin, 2021). BPOM reports that there are 14 types or 27,299 cosmetics sold online in North Jakarta and as many as 26 types or 188,395 cosmetics found in South Jakarta with a search range of one to two months (BPOM, 2020). Based on sampling and testing conducted in July 2020-September 2021, 18 cosmetic products contain prohibited and dangerous ingredients which are dominated by Hydroquinone and prohibited dyes, namely Red K3 and Red K10 (BPOM, 2021).

The 2011 BPOM regulation explains that Rhodamine B is prohibited from being used as food coloring and cosmetics because it causes irritation if it is exposed to the eyes, skin, poisoning, impaired liver function and cancer (Herdini and Wahyidiana, 2019). The increasing need for cosmetics and technological developments support the expansion of sales marketing in an easy and practical way. The electronic system or call the marketplace is one of the media providers of buying and selling goods or services. So many may sell illegal products or contain prohibited and dangerous ingredients.

Several previous studies related to cosmetic preparations containing Rhodamine B have been carried out, such as in the study conducted by Jusnita and Nandu (2017) of 25 samples there were 4 positive samples of Rhodamine B with a standard R_f value of Rhodamine B of 0.8 samples. D2 0.8; D3 0.8; E5 0.8; and E6 0.8. The mobile phase consists of a mixture of ethyl acetate: methanol: [ammonia-water] (15:3:3). Research conducted by Afriyeni and Utari (2016) of the 5 test samples contained 1 positive sample of Rhodamine B with an R_f value of 0.66 and a standard R_f value of 0.67. The mobile phase mixture used ethyl acetate: methanol: ammonia 9% (15:3:3). Research by Nafiq *et al* (2020) of 9 samples of 4 samples resulted in positive results. Rhodamine B has an R_f value of 0.82, B code sample is 0.76, D sample is 1, E code sample is 0.74 and H has an R_f value of 0.72. The eluent used was a mixture of n-butanol: ethyl acetate: ammonia ratio (55:20:25).

This study takes eyeshadow samples in marketplace X because no one has examined samples from marketplace X. Based on this description, researchers are interested in conducting research to determine the presence of Rhodamine B in eyeshadow sold in marketplace X.

METHOD

Tools and Materials

Analytical Chromatography silica gel GF254 Merck, CAMAG UV cabinet dual aveleght 254 and 366, CAMAG Scientific Twin Trough Chamber for 20×10 cm Plates, Analytical Scales (Ohaus), Oven (IKA) 100 mL measuring cup (Pyrex), 10 mL measuring cup (Pyrex), 100 mL volumetric flask (IWAKI), 25 mL volumetric flask (IWAKI), 10 mL volumetric flask (IWAKI), Socorex 2—20μL Micropipette, Stirring Rod, Watch Glass, Funnel, Filter Paper, Volume Pipette, Dropper Pipette, Eyeshadow samples sold in marketplace X, Rhodamine B (Sigma), Methanol (Pro analysis), Aquades, Ethyl acetate (Pro analysis), Ammonia (Pro analysis), Hydrochloric acid (Pro analysis), N-butanol (Pro analysis), Ethanol (Pro analysis).

Qualitative Analysis

Preparation of Test Solution

A total of 100 mg of the sample was added 1 drop of 4M HCl. Then add 5 mL of methanol (Pro analysis) and homogenize. Then the sample solution was separated with filter paper and put the filtrate into the ampoule (Fauziah *et al.*, 2020).

Preparation of standard Solution

A total of 10 mg of standard Rhodamine B was weighed and then dissolved in a 100 mL volumetric flask with methanol (Pro analysis) up to the calibration limit mark. The solution was shaken homogeneously (100 g/mL) (Elfasyari *et al.*, 2020).

Identification of Rhodamine B using TLC

To obtain chromatographic results with round/elliptical and tailless spots in the stationary phase, prior to spotting the plate, optimization of the mobile phase with various compositions and concentrations was carried out:

1. n-Butanol : Ethyl Acetate : Ammonia (55:20:25) (Nafiq *et al.*, 2020)
2. Ethyl acetate: Methanol : Ammonia (15:6:3) (Riyanti *et al.*, 2018)
3. Ethyl acetate: Methanol : Ammonia (15:3:3) (Afriyeni and Utari, 2016)
4. Ethyl acetate : Methanol : Ammonia + Water ratio (15:3:3) (Jusnita and Nandu, 2017)
5. Ethyl acetate: Methanol: Ammonia (25:6:1) (Ratnaningtyas, 2013)
6. n-butanol : Ethanol : Aquadest (20:12:15) (Fatimahet *et al.*, 2016)

Rhodamine B standard spotting and eyeshadow samples on a thin layer chromatography plate by:

1. Using a TLC plate measuring 4 x 10 cm, it was previously dried by heating it in an oven at 100 °C for 30 minutes.
2. The parent solution and the test solution (sample) were spotted using a micropipette as much as 3μL with a distance of 1 cm from the bottom of the plate and a distance between the spots of 1 cm.
3. Dry the spots using a hairdryer.
4. The TLC plate containing the sample is dipped into a chamber with the mobile phase in it having been pre-saturated using filter paper. The plate was allowed to stand until completely eluted and removed, dried at room temperature.
5. Observe the color of the stain visually and under ultraviolet light. If the color of the stain is pink and below UV 254 and 366 nm it fluoresces yellow or orange, it proves the presence of Rhodamine B dye, the Rf value is calculated (Nafiq *et al.*, 2020).
6. The plate with a positive sample of Rhodamine B was sharpened by a color reaction using an HCl spray reaction, it will turn pink if it reacts. Then calculate the Rf value that appears on the plate that has been sprayed (Annas and Sapriyanto, 2017).

Data analysis

Data analysis in this study used descriptive statistical tests by processing the Rf value data and sample resolution in the form of images and tables. The results of data processing in the form of images and tables are described or interpreted to clarify information regarding the description of Rhodamine B content

in eyeshadow preparations sold in marketplace X.

RESULTS

The results of the optimization of the mobile phase in this study were carried out by measuring and calculating the Rf value and resolution in the variation of the mobile phase mixture as shown in table 1.

Table 1. Optimization of Mobile Phase Mixture

(P1) mobile phase n-butanol : Ethanol : Aquadest (20:12:15)				
Sample	Rf Value	Difference Rf	Resolution	Description
Rhodamine B	0,7	-	-	Homogeneous mobile phase
I	0,74	0,04	9,37	
II	0,73	0,03	6,43	
III	0,74	0,04	8,04	
(P2) mobile phase Ethyl acetate : Methanol : Ammonia (15:6:3)				
Sample	Rf Value	Difference Rf	Resolution	Description
Rhodamine B	0,75	0,04	2,33	Homogeneous mobile phase
I	0,75	0,05	2,29	
II	0,75	0,04	1,5	
III	0,76	0,04	1,5	
(P3) fase gerak Etil asetat : Metanol : Amoniak (25:6:1)				
Sample	Rf Value	Difference Rf	Resolution	Description
Rhodamine B	0,41	0,08	1,20	Homogeneous mobile phase
I	0,41	0,08	1,44	
II	0,40	0,09	0,93	
III	0,40	0,06	1,11	
(P4) mobile phase Ethyl acetate : Methanol : Ammonia (25:6:1)				
Sample	Rf Value	Difference Rf	Resolution	Description
Rhodamine B				The mobile phase forms 2 inhomogeneous layers
I	-	-	-	
II				
III				
(P5) mobile phase Ethyl acetate : Methanol : Ammonia + water (15:3:3)				
Sample	Rf Value	Difference Rf	Resolution	Description
Rhodamine B				The mobile phase forms 2 inhomogeneous layers
I	-	-	-	
II				
III				
(P6)mobile phase Ethyl acetate : Methanol : Ammonia (15:3:3)				
Sample	Rf Value	Difference Rf	Resolution	Description
Rhodamine B				The mobile phase forms 2 inhomogeneous layers
I	-	-	-	
II				
III				

The optimization of the mobile phase in this study also shows the shape and color of the stain from the eyeshadow sample. The results of the stains seen visually, under UV light 254 and 366 nm on the TLC plate can be shown in Figure 1.

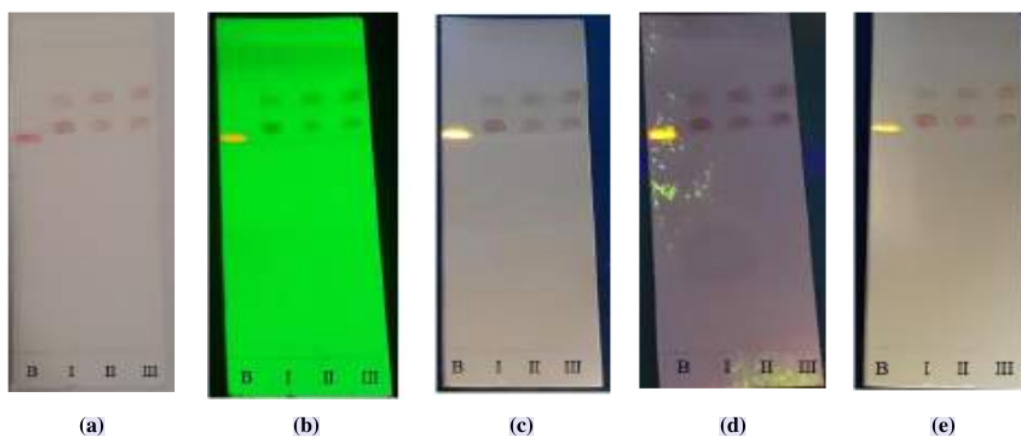


Figure 1. Spots of mobile phase n-butanol : Ethanol : Aquadest (20:12:15)

Information : (a) Visual

(b) under UV light 254

(c) under UV light 366

(d) under UV light 254 after spraying HCl

(e) under UV light 366 after spraying HCl

B : Standard

I : Replication 1

II : Replication 2

III: Replication 3

Figure 2 shows the results of the stains seen visually on the TLC plate.

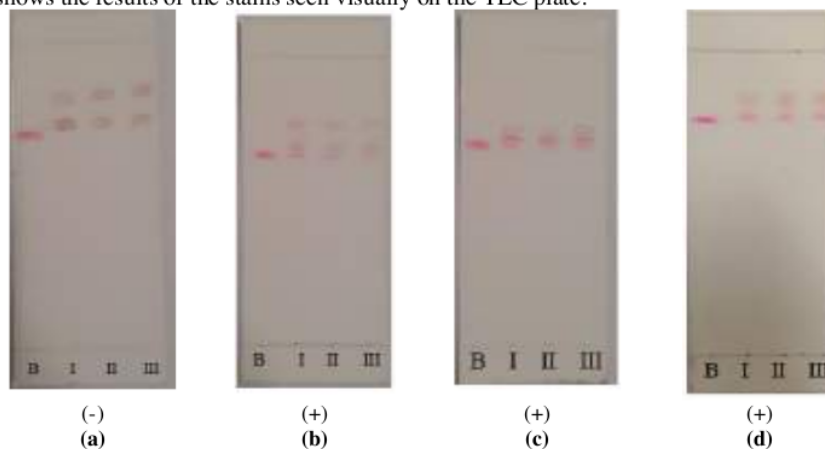


Figure 2. Visually positive sample stains

Information : (a) Negative sample

(b) Sample 4

(c) Sample 8

(d) Sample 9

B : Standard

I : Replication 1

II : Replication 2

III: Replication

Figure 2 shows visually the stains are seen based on the shape and color of the stain, as for the positive results of samples containing Rhodamine B indicated by a bright pink color and the shape of the stain is round/ellipse. Meanwhile, the negative results of samples containing Rhodamine B are shown without a red color bright young.

The positive and negative results on samples containing Rhodamine B in UV 254 light can be seen in Figure 3.

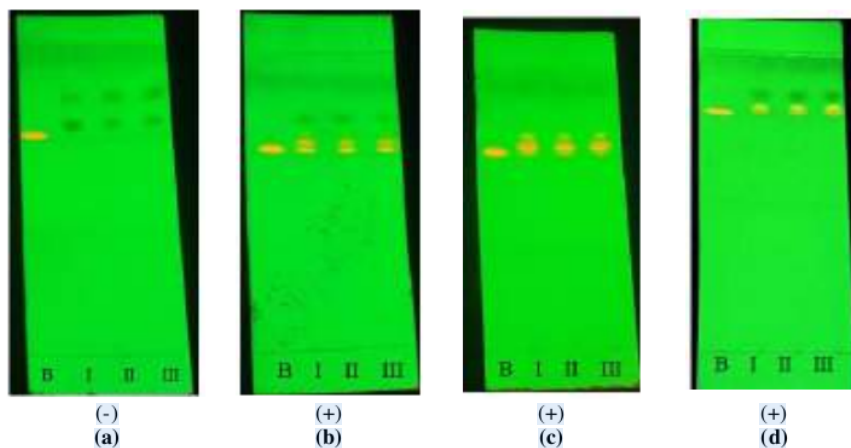


Figure 3. Spots of positive sample stains under UV light 254

Information : (a) Negative sample
 (b) Sample 4
 (c) Sample 8
 (d) Sample 9
 B: Standard
 I: Replication 1
 II: Replication 2
 III: Replication

Figure 3 shows the stains on the sample that are positive for Rhodamine B under UV 254 light, which looks yellow or orange fluorescence and the shape of the stain is round/elliptical, while the spots on the sample that are negative for Rhodamine B are indicated by stains that do not fluoresce yellow or orange.

The positive and negative results on samples containing Rhodamine B in UV 366 light can be seen in Figure 4.

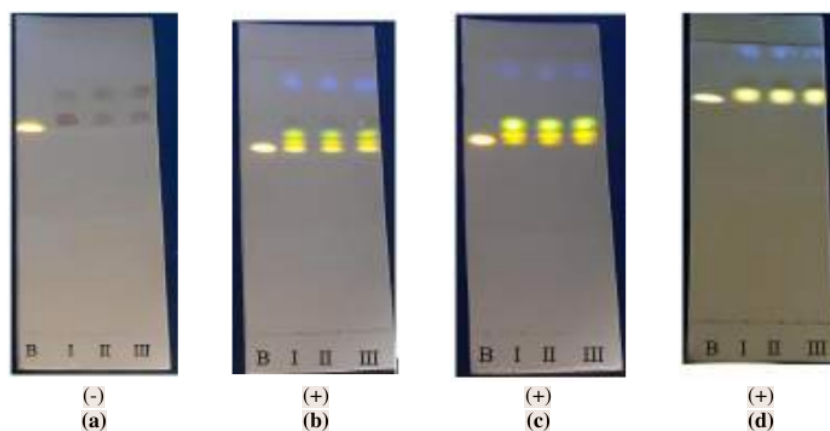


Figure 4. Spots of positive sample stains under 366 . UV light

Information : (a) Negative sample
 (b) Sample 4
 (c) Sample 8
 (d) Sample 9

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B : Standard

II : Replication 2

I : Replication 1

III: Replication

Figure 4 shows that the stains on the sample that are positive for Rhodamine B under UV 366 light appear to have yellow or orange fluorescence and the shape of the stain is round/elliptical, while the spots on the sample that are negative for Rhodamine B are indicated by stains that do not fluoresce yellow or orange.

The results of the calculation of the R_f and Resolution values in this study are shown in table 2.

Table 2. Qualitative Test Results Eyeshadow Samples using the mobile phase n-butanol : Ethanol : Aquadest (20:12:15)

Rep. Sample	Visual	UV 254 nm	UV 366 nm	R _f value	R _s value	Difference R _f	Desc
1	Rhodamine B	Pink	Yellow fluorescence	0,7	-	-	(-)
	I	Faded pink	Not fluorescence	0,74	9,37	0,04	
	II	Faded pink	Not fluorescence	0,73	6,43	0,03	
	III	Faded pink	Not fluorescence	0,74	8,04	0,04	
2	Rhodamine B	Pink	Yellow fluorescence	0,68	-	-	(-)
	I	Faded red	Not fluorescence	0,73	3,53	0,04	
	II	Faded red	Not fluorescence	0,73	2,44	0,04	
	III	Faded red	Not fluorescence	0,83	3,05	0,15	
3	Rhodamine B	Pink	Yellow fluorescence	0,69	-	-	(-)
	I	Faded red	Not fluorescence	0,73	0	0,04	
	II	Faded red	Not fluorescence	0,73	0	0,04	
	III	Faded red	Not fluorescence	0,74	0	0,05	
4	Rhodamine B	Pink	Yellow fluorescence	0,65	-	-	(+)
	I	Pink	Yellow fluorescence	0,65	2,69	0,00	
	II	Pink	Yellow fluorescence	0,64	4,01	0,01	
	III	Pink	Yellow fluorescence	0,64	2,52	0,01	
5	Rhodamine B	Pink	Yellow fluorescence	0,73	-	-	(-)
	I			-	-	-	
	II			-	-	-	
	III			-	-	-	
6	Rhodamine B	Pink	Yellow fluorescence	0,66	-	-	(-)

7	I	Faded red	Not fluorescence	Not fluorescence	0,7	7,03	0,04	(-)
	II	Faded red	Not fluorescence	Not fluorescence	0,7	7,03	0,04	
	III	Faded red	Not fluorescence	Not fluorescence	0,71	13,02	0,05	
	Rhodamine B	Pink	Yellow fluorescence	Not fluorescence	0,65	-	-	
	I	Pink	Not fluorescence	Fluorescent orange	0,73	2,03	0,08	
	II	Pink	Not fluorescence	Fluorescent orange	0,74	2,02	0,09	
	III	Pink	Not fluorescence	Fluorescent orange	0,74	2,02	0,09	
	Rhodamine B	Pink	Yellow fluorescence	Fluorescent orange	0,68	-	-	
	I	Pink	Yellow fluorescence	Fluorescent orange	0,67	4,01	0,02	
	II	Pink	Yellow fluorescence	Fluorescent orange	0,66	4,02	0,01	
8	III	Pink	Yellow fluorescence	Fluorescent orange	0,66	2,69	0,01	(+)
	Rhodamine B	Pink	Yellow fluorescence	Fluorescent orange	0,78	-	-	
	I	Pink	Yellow fluorescence	Fluorescent orange	0,79	2,03	0,01	
	II	Pink	Yellow fluorescence	Fluorescent orange	0,79	2,03	0,01	
	III	Pink	Yellow fluorescence	Fluorescent orange	0,79	2,03	0,01	
9	Rhodamine B	Pink	Yellow fluorescence	Not fluorescence	0,75	-	-	(-)
	I	-	-	-	-	-	-	
	II	-	-	-	-	-	-	
10	III	-	-	-	-	-	-	(-)

DISCUSSION

In this study, the sample used was eyeshadow cosmetic preparations sold in marketplace X by optimizing the mobile phase using the thin layer chromatography method. Eyeshadow samples were taken from stores with star criteria and star+ is a store that has the best performance and service and sales. Observation of the stain for the thin layer chromatography method can be seen from the shape of the stain which is round/elliptical. Optimization of the mobile phase is carried out to obtain accurate measurement results. Obtaining a mobile phase with a suitable mixture of solutions for the separation of samples and other compounds results in the shape of the stain and the color of the stain is clear. Optimization of the mobile phase is determined from the physicochemical properties provided that it has sufficient purity, stability, low viscosity, vapor pressure that is not too low or too high, and the lowest possible toxicity.

The variation in concentration of the solvent mixture used affects the polarity of the mobile phase. A mixture of mobile phases consisting of very polar and very non-polar solvents will produce a mobile phase that is less mixed or demixed, causing the stains that appear to be uncalculated, the Rf value is not calculated, the stain is tailless, not round, and wide (Wulandari, 2011). The immiscibility of the mobile phase is due to the difference in polarity between the mobile phase and the sample which results in the stain not being completely eluted, the closer the polarity of the mobile phase to the compound to be tested, the better the separation, as in principle, like dissolved like. The nature of Rhodamine B affects the selection of the mobile phase because Rhodamine B has a carboxyl group with a lone pair of electrons and an amine group so that

intermolecular hydrogen bonds will form with a solvent that has a polar nature, so that Rhodamine B compounds will easily dissolve in polar solvents, so a mixture of mobile phases is used. a mixture of solutions with polar properties that will produce a good separation of Rhodamine B with other compounds (Nafiq *et al.*, 2020).

The selected mobile phase will be inserted into the chamber for saturation, the type of chamber used is a twin trough chamber which has better advantages compared to chamber N and can control saturation and humidity in the chamber. Saturation using filter paper for approximately 15 minutes, on each side of the twin trough chamber to control the process of separating compounds that are very susceptible to changes in humidity. The use of a saturated chamber will have a lower Rf value than an unsaturated chamber with the same handling conditions (Wulandari, 2011). The stationary phase uses silica GF 254 because on the surface of the silica gel it has Si-OH (silanol) and Si-O-Si (siloxane) groups. The stationary phase was dried first by heating at a temperature of 100°C for 30 minutes to remove the water content that was absorbed in the plate. Improper spotting will result in stains with scattered, tailed, non-round/elliptical spots and produce double peaks. The sample volume is at least 0.5 µL – 10 µL (Wulandari, 2011).

Methanol is used as an organic solvent that has polar properties and a low boiling point that will dissolve organic substances that have polar properties well. The use of HCl serves to destroy other compounds contained in the eyeshadow sample and to clarify the red color of Rhodamine B and HCl can stabilize the Rhodamine B compound contained in the eyeshadow sample, so that it does not turn into a neutral form from the ionized form (Nafiq *et al.*, 2020). To obtain a clear solution without disturbing substances, a filtering process is carried out.

In this study, to determine the appropriate mobile phase indicated by the shape and color of the stain on the TLC plate of the eyeshadow sample visually, under UV light 254 and 366 nm, the Rf value, the difference in the Rf value, the resolution value and the homogeneity of the solvent mixture were calculated as mobile phase. The Rf value was used to see the migration of compounds on the TLC plate. The difference in Rf is used to determine the distance between the Rhodamine B standard and the stain that is declared positive and the Resolution value is used to see the ability of the analyte to separate Rhodamine B compounds from other compounds in the eyeshadow sample. The results of the optimization of the mobile phase selected a mixture of (P1) n-butanol: Ethanol: Aquadest (20:12:15) with a clear, homogeneous solution, producing stains with an Rf value that entered the requirement range of 0.2 – 0.8, the difference between the Rf standard of Rhodamine B with sample 0.2 and Resolution value 1.5 compared to other mobile phase mixtures P1 has a better resolution or separation value and has a clear and homogeneous solution mixture. The results of the study can be seen in table 1.

Stains containing Rhodamine B visually showed stains parallel to the Rhodamine B standard having a light pink color. The results of stains that do not contain Rhodamine B are shown by the color of the stains being the same as the color of the sample solution and not bright pink. Observation of stains under UV light 254 and 366 resulted in yellow or orange fluorescence stains, samples with non-fluorescent stains showed no Rhodamine B content in the sample. HCl spraying is used to clarify the color of stains from samples that are positive with stains that fluoresce under UV 254 and 366 yellow or orange, spraying can also indicate new stains that appear (Nafiq *et al.*, 2020). The results of the optimization of the mobile phase mixture which were observed visually under UV 254 and 366 are shown in Figure 1.

The test results from ten samples of eyeshadow sold in marketplace X, there are three samples with positive results indicated by samples 4, 8, and 9 in Figure 2, Figure 3, and Figure 4. The stains produce a round/elliptical shape with no tail. The stains visually observed were bright pink and yellow fluorescence under UV 254 light and orange under UV 366 light. Optimal results will be obtained if spotting the sample on the plate with the smallest and narrowest spot size possible, the more sample volume that is applied will decrease the sample resolution value. A good separation is obtained if a thin, tall, and slender peak is obtained (Rohman, 2020). The results of the qualitative test using the mobile phase of n-butanol : Ethanol : Aquadest (20:12:15) are shown in table 2. Tests on samples 1, 2, 3, 5, 6, 7, and 10 showed negative test results observed from the shape of the stains resulting in round/elliptical stains and no tails. Visually observed, it did not show a bright pink stain on the stain, and under UV light 254 nm and 366 nm did not show a yellow or orange fluorescence stain which resulted in the sample not showing the presence of Rhodamine B compounds. Sample 1 showed an Rf value that entered the range requirements for qualitative analysis. 0.2 – 0.8 and the terms value Rs 1.5.

CONCLUSION

In this study, it can be concluded that Rhodamine B content analysis by optimizing the mobile phase using the thin layer chromatography method on eyeshadow preparations sold in marketplace X, namely:

1. Spots with a positive result, visually showed a bright pink color similar to the color of the standard Rhodamine B stain. Observations under UV 254 and 366 with positive samples resulted in yellow or orange fluorescence stains. HCl spray was used to clarify the color of stains containing Rhodamine B.
2. The optimal mobile phase is the mobile phase with a mixture of n-butanol: Ethanol: Aquadest ratio (20:12:15).
3. The ten eyeshadow samples studied were three samples that showed positive results containing Rhodamine B.

ACKNOWLEDGMENTS

The author would like to thank STIKes Mitra Keluarga Bekasi for the support during the author's research.

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