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

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Abstract

Introduction: Blush on is a cosmetic to color the cheeks with various colors such as red, pink, to peach. The use of blush on is often misused by adding harmful dyes such as Rhodamine B. The impact of Rhodamine B can cause skin irritation and respiratory tract, and it is a carcinogen if continuously exposed and accumulates in the body; therefore, it is necessary to research the detection of Rhodamine B content in blush on cosmetic sold on Marketplace X. **Method:** Identification of Rhodamine B by optimizing the mobile phase using the Thin Layer Chromatography method. The design of this research is experimental with a purposive sampling technique and samples are taken according to the criteria: Red and Pink color, imported products, solid powder with a price range of Rp. 5000–25,000,does not have a notification number from National Agency of Drug and Food Control. Data analysis using Rf and Rs values obtained. **Results:** 12 samples of blush on using TLC method with optimal mobile phase mixture of n- Butanol: Ethanol 96%: Aquadest (20:12:15), Samples that were positive for Rhodamine B produced round/elliptical and tailless stains. Visually it shows a bright pink color, and under UV light, 254 nm and 366 nm fluoresces yellow or orange. Sample A3 has an average R_f value 0.79 and Rs 3.02. Sample A4 average R_f value 0.77 and Rs 2.35. Sample A6 has an average R_f value of 0.68 and an Rs of 2.02. Sample A7 has an average R_f value of 0.76 and an Rs of 2.19. Sample A8 average R_f value of 0.69 with an Rs value of 2.69. Sample A11 has an average R_f value of 0.68 and an Rs of 4.23, and sample A12 has an average R_f value of 0.69 with an average Rs value of 1.73. **Conclusion:** 7 on of 12 samples blush on contain Rhodamin B

Key words : *Mobile phase, Cosmetic, Rhodamine B, Blush on, Thin Layer Chromatography*

INTRODUCTION

Cosmetic products in Indonesia are currently increasing rapidly, due to the many cosmetic products sold inthe market and Marketplace at affordable prices ranging from well-known brands to illegal cosmetic products so that many consumers are interested in buying cosmetic products without knowing the ingredients contained in the cosmetics. One of the cosmetics that is often used by women is blush on. Blush on is a cosmetic to color the cheeks in the form of cream, liquid, powder or gel with various colors such as red, pink, to peach. Blush contains high concentrations of dyes and pigments, zinc oxide and titanium dioxide (Andayani et al., 2019). The use of blush is often misused by adding harmful dyes such asRhodamine B. Rhodamine B is commonly used as a textile dye, dye for cotton, bark, paper, wool, nylon and varnish, in the form of a greenish crystalline powder, in dissolved form at high concentrations. purplish red and at low concentrations bright red (Putri et al., 2017). The side effects of rhodamine B can cause skin irritation, and are carcinogenic if continuously exposed and accumulated in the body (Nanda and Darayani, 2018). According to BPOM Regulation Number 23 of 2019 concerning Technical Requirements for Cosmetic Ingredients, it is stated that Rhodamine B is prohibited from being used for cosmetic additives, so it is necessary to conduct research on the content of Rhodamine B in blush on cosmetic sold in marketplace X using the Thin Layer chromatography method.

METHOD

Research Tools and Materials

The tools used in this study were GF254 silica gel plate, Analytical balance (Ohaus), 100 mL measuring cup (Herma), 100 mL volumetric flask (Pyrex), 25 mL volumetric flask (Pyrex), 10 mL volumetric flask (Pyrex). 10 mL Beaker glass (Iwaki), Oven (Ika), UV Lamp (CAMAG), Chamber (CAMAG), Stirring Rod, Funnel (Pyrex), Volume pipette (Iwaki), Dropper pipette (Pyrex), Micropipette Socorex 2-20 µl and filter paper, while the materials used included samples of blush sold in Marketplace X, Rhodamin B Standard (Sigma), Methanol p.a (Merck), Aquadest, Ethylacetate p.a (Merck), Ammonia p.a (Merck), n- Butanol p.a (Merck), Hydrochloric acid p.a.

Analysis Qualitativ:

Preparation of standard solution

Dissolve 10 mg of Rhodamine B standard into a 100 mL volumetric flask using methanol p.a, stir homogeneously, then add methanol p.a to the mark of the volumetric flask and stir until homogeneous (100 ppm) (Elfasyari et al., 2020).

Preparation of standard solution

A total 500 mg of the blush sample and put it in a beaker glass then add 4 drops of 4 M HCl and enter 2 mLof methanol p.a then stir homogeneously and then enter it into a 25 mL volumetric flask and homogenize (Elfasyari et al., 2020). Add methanol p.a ad 25 mL, then stir homogeneously and filter the solution using filter paper (Elfasyari et al., 2020).

Mobile Phase Optimization

Optimization is done by spotting a test solution containing Rhodamine B and a comparison solution, namely Rhodamine B standard solution. Optimization is carried out with various types of solution mixture compositions and with various concentrations that have been carried out by previous researchers as follows:

1. Ethyl acetate: Methanol: Ammonia (25:6:1) (Ratnaningsih, 2013)
2. n-Butanol: Ethanol 96%: Aquadest (20:12:15) (Fatimah et al., 2016)
3. Ethyl acetate: Methanol: Ammonia (15:6:3) (Riyanti et al., 2018)
4. Ethyl acetate: Methanol: Ammonia + Aquadest (15:3:3) (Justina and Nandu, 2016)
5. Ethyl acetate: Methanol: Ammonia (15:3:3) (Afriyeni and Utari, 2016)
6. n-Butanol: Ethyl acetate: Ammonia (55:20:25) (Yuniarto and Rosalina, 2019)

Qualitative Analysis of Thin Layer Chromatography Method

1. Activate the TLC plate measuring 4x10 cm by heating it for 30 minutes in the oven at 100°C.
2. The Standard solution and the test solution were spotted as much as 3 l using a micropipette on the plate with a distance of 1 cm from the bottom of the plate and 1 cm for the distance between the stains.
3. Allow the plate containing the sample to dry for a while then insert the plate into a chamber that has been saturated with the selected mobile phase.
4. Remove the plate from the fully eluted vessel.
5. Observations were made on the color of the stain on the plate visually (pink) and under UV lamps of 254 nm and 366 (yellow or orange fluorescence), this indicated the presence of Rhodamine B dye. The Rf value was calculated.
6. The klt plate was sharpened with HCl spray reagent (producing a pink color) (Tjuana et al., 2021).

RESULTS

In this study, apart from looking at the shape and color of the stain on the TLC plate, it also calculated theRf value, Rf difference, Rs value and homogeneity of the mobile phase solvent mixture. The results of theRf and Rs values in this study are shown in table 1.

Table 1. Mobile Phase Optimization

(MPI) Mobile Phase Etil asetat : Methanol : Ammoniak (25:6:1) (PI:4,71)				
Sample	R_f value	R_f value difference	Rs (Resolution)	Description
Rhodamine B	0,34	TD	TD	Homogeneous mobile phase
I	0,34	0	3,05	
II	0,34	0	2,05	
III	0,34	0	2,05	
(MPII) Mobile Phase n-Butanol : Ethanol 96 % : Aquadest (20:12:15) (PI:6,22)				
Sample	R_f value	R_f value difference	Rs (Resolution)	Description
Rhodamine B	0,68	TD	TD	Homogeneous mobile phase
I	0,68	0	3,35	
II	0,68	0	5,03	
III	0,69	0,01	3,03	
(MPIII) Mobile Phase Etil asetat : Methanol : Ammoniak (15:6:3) (PI:5,3)				
Sample	R_f value	R_f value difference	Rs (Resolution)	Description
Rhodamine B	0,88	TD	TD	Homogeneous mobile phase
I	0,88	0	3,53	
II	0,88	0	4,03	
III	0,88	0	2,03	
(MPV) Mobile Phase Etil asetat : Methanol : Ammoniak + Aquadest (15:6:3) (PI:5,3)				
Sample	R_f value	R_f value difference	Rs (Resolution)	Description
Rhodamine B	ND	ND	ND	The mobile phase is not homogeneous to form 2 layers
I				
II				
III				
(MPV) Mobile Phase Etil asetat : Methanol : Ammoniak (15:6:3) (PI:5,32)				
Sample	R_f value	R_f value difference	Rs (Resolution)	Description
Rhodamine B	ND	ND	ND	The mobile phase is not homogeneous to form 2 layers
I				
II				
III				
(MPVI) Mobile Phase n-Butanol : Etil asetat : Ammoniak (55:20:25) (PI:5,55)				
Sample	R_f value	R_f value difference	Rs (Resolution)	Description
Rhodamine B	ND	ND	ND	The mobile phase is not homogeneous to form 2 layers
I				
II				
III				

Information

MP	: Mobile phase	I	: Replication 1
ND	: Not detected	II	: Replication 2
PI	: Polarity index	III	: Replication 3

Table 1 shows the results of the optimization of the mobile phase, the mixture (MPII) of n-Butanol: ethanol 96%: aquadest (20:12:15) best met the criteria, namely a clear and homogeneous solution with an Rf value range of 0.2-0. 8 the difference between the R_f values of the sample with R_f Rhodamin B 0.2 and the value of Rs 1.5 compared to other mobile phase mixtures, the FGII mixture has a good Rf value, a good value of Rs or separation and produces a clear and homogeneous solution mixture.

The results of the optimization of the mobile phase in this study showed the shape and color of the stain onthe blush sample. The results of the stains seen visually on the TLC plate can be shown in Figure 1.

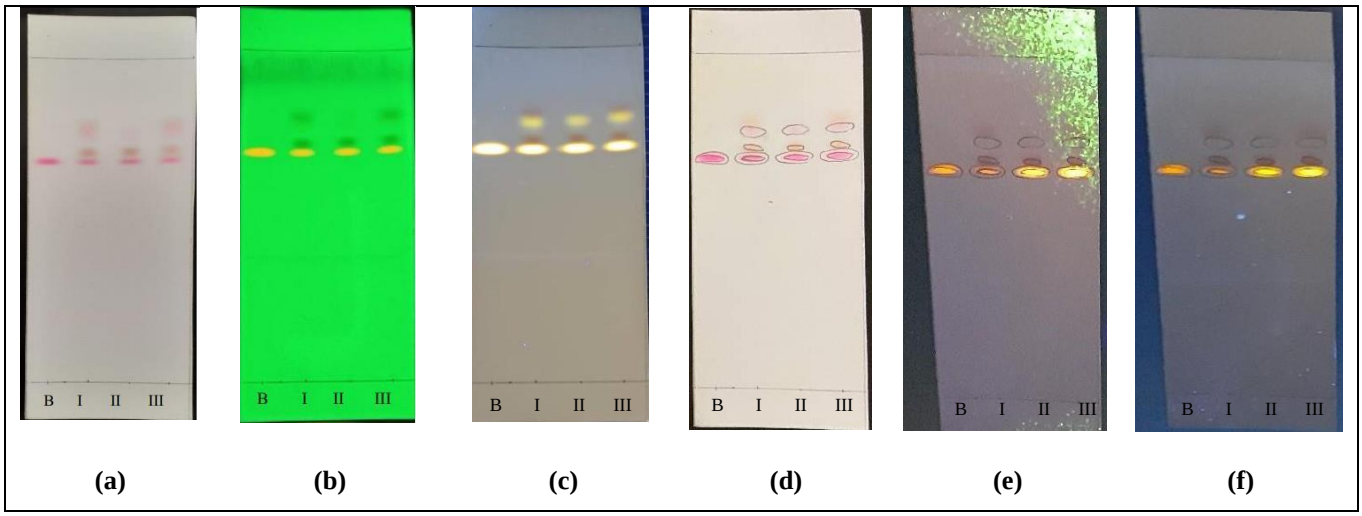

Figure 1. Spots of mobile phase n-Butanol : Ethanol : Aquadest (20:12:15)

Information : (a) Visual
(b) Under UV light 245 nm
(c) Under UV light 366 nm
(d) Visual after sparying HCl
(e) Under UV light 254 nm after spraying HCl
(f) Under UV light 366 nm after spraying HCl

B: Standard
I : Replication 1
II : Replication 2
III : Replication 3

Figure 1 shows stain under 366 nm UV light after being sprayed with 4 M HCl based on the shape and color of the stain, a positive result of a blush sample containing Rhodamine B is indicated by a yellow or orange fluorescence stain and a round/elliptical shape of the stain, while the spots are stained with yellow or orange fluorescence. stains on samples that do not contain Rhodamine B are indicated by stains that do not fluoresce yellow or orange.

Table 2. Results of Cosmetic Blush Samples using mobile phase n-butanol : Ethanol 96 % : Aquadest (20:12:15)

Rep.	Sample	Visual color	UV 254 nm	UV 366 nm	Rf value	Rf value difference	Average difference Rf	Rs value	Average Rs value	Result
A1	Rhodamine B	Pink	Orange fluorescence	Orange fluorescence	0.70	ND	ND	ND	ND	(-)
	I	Faded pink	non-fluorescent	non-fluorescent	0.73	0.03	0.03	4.01	3.75	
	II	Faded pink	non-fluorescent	non-fluorescent	0.73	0.03		3.23		
	III	Faded pink	non-fluorescent	non-fluorescent	0.73	0.03		4.03		
A2	Rhodamine B	Pink	Orange fluorescence	Orange fluorescence	0.74	ND	ND	ND	ND	(-)
	I	Faded pink	non-fluorescent	non-fluorescent	0.75	0.01	0.01	9.03	9.03	
	II	Faded pink	non-fluorescent	non-fluorescent	0.75	0.01		9.03		
	III	Faded pink	non-fluorescent	non-fluorescent	0.75	0.01		9.03		
A3	Rhodamine B	Pink	Orange fluorescence	Orange fluorescence	0.75	ND	ND	ND	ND	(+)

	I	Pink	Orange fluorescence	Orange fluorescence	0.79	0.04		3.02		
	II	Pink	Orange fluorescence	Orange fluorescence	0.79	0.04	0.04	3.02	3.02	
	III	Pink	Orange fluorescence	Orange fluorescence	0.79	0.04		3.02		
A4	Rhodamine B	Pink	Orange fluorescence	Orange fluorescence	0.75	ND	ND	ND	ND	
	I	Pink	Orange fluorescence	Orange fluorescence	0.77	0.02		2.01		
	II	Pink	Orange fluorescence	Orange fluorescence	0.77	0.02	0.02	2.01	2.35	(+)
	III	Pink	Orange fluorescence	Orange fluorescence	0.78	0.03		3.03		
A5	Rhodamine B	Pink	Orange fluorescence	Orange fluorescence	0.71	ND	ND	ND	ND	
	I	Brick red	non-fluorescent	non-fluorescent	0.76	0.04		4.38		
	II	Brick red	non-fluorescent	non-fluorescent	0.78	0.06	0.06	4.04	4.42	(-)
	III	Brick red	non-fluorescent	non-fluorescent	0.79	0.08		4.84		
A6	Rhodamine B	Pink	Orange fluorescence	Orange fluorescence	0.66	ND	ND	ND	ND	
	I	Pink	Orange fluorescence	Orange fluorescence	0.68	0.02		2.02		
	II	Pink	Orange fluorescence	Orange fluorescence	0.68	0.02	0.02	2.02	2.02	(+)
	III	Pink	Orange fluorescence	Orange fluorescence	0.68	0.02		2.02		
2A7	Rhodamine B	Pink	Orange fluorescence	Orange fluorescence	0.75	ND	ND	ND	ND	
	I	Pink	Orange fluorescence	Orange fluorescence	0.77	0.02		1.51		
	II	Pink	Orange fluorescence	Orange fluorescence	0.76	0.01	0.01	2.03	2.19	(+)
	III	Pink	Orange fluorescence	Orange fluorescence	0.76	0.01		3.03		
A8	Rhodamine B	Pink	Orange fluorescence	Orange fluorescence	0.68	ND	ND	ND	ND	
	I	Pink	Orange fluorescence	Orange fluorescence	0.69	0.01		2.02		
	II	Pink	Orange fluorescence	Orange fluorescence	0.69	0.01	0.01	3.02	2.69	(+)
	III	Pink	Orange fluorescence	Orange fluorescence	0.69	0.01		3.02		
A9	Rhodamine B	Pink	non-fluorescent	non-fluorescent	0.66	ND	ND	ND	ND	
	I	Faded brick red	non-fluorescent	non-fluorescent	0.68	0.02		11.08		
	II	Faded brick red	non-fluorescent	non-fluorescent	0.68	0.02	0.02	10.57	10.4	(-)
	III	Faded brick red	non-fluorescent	non-fluorescent	0.69	0.03		9.54		

A10	Rhodamine B	Pink	non-fluorescent	non-fluorescent	0.68	ND	ND	ND	ND	
	I	Faded brick red	non-fluorescent	non-fluorescent	0.70	0.02		4.54		
	II	Faded brick red	non-fluorescent	non-fluorescent	0.71	0.03	0.03	4.04	4.54	(-)
	III	Faded brick red	non-fluorescent	non-fluorescent	0.71	0.03		5.05		
A11	Rhodamine B	Pink	Orange fluorescence	Orange fluorescence	0.69	ND	ND	ND	ND	
	I	Pink	Orange fluorescence	Orange fluorescence	0.68	0.01		5.01		
	II	Pink	Orange fluorescence	Orange fluorescence	0.68	0.01	0.01	2.68	4.23	(+)
	III		Orange fluorescence	Orange fluorescence	0.68	0.01		5.01		
A12	Rhodamine B	Pink	Orange fluorescence	Orange fluorescence	0.69	ND	ND	ND	ND	
	I	Pink	Orange fluorescence	Orange fluorescence	0.69	0		1.51		
	II	Pink	Orange fluorescence	Orange fluorescence	0.69	0	0	2.03	1.73	(+)
	III	Pink	Orange fluorescence	Orange fluorescence	0.69	0		1.68		

Table 2. Shows the results of cosmetic blush samples using a mixture of mobile phase n-Butanol : 95% Ethanol : Aquadest (20:12:15) resulting from 12 samples there are 7 positive samples shown in the sample with the code A3; A4; A6; A7; A8; A11 and A12 with stain criteria visually bright pink and fluoresces yellow or orange under UV light at 254 nm and 366 nm, respectively. The Rf value is in the price range of 0.2-0.8 with the difference in the Rf value between the Rhodamine B standard and the sample of 0.2 and the value of Rs 1.5.

The results of the sample using the mobile phase n-Butanol: Ethanol 96%: Aquadest (20:12:15) in this study showed the shape and color of the stain on the blush sample. The results of the stains seen visually on the TLC plate can be shown in Figure 2.

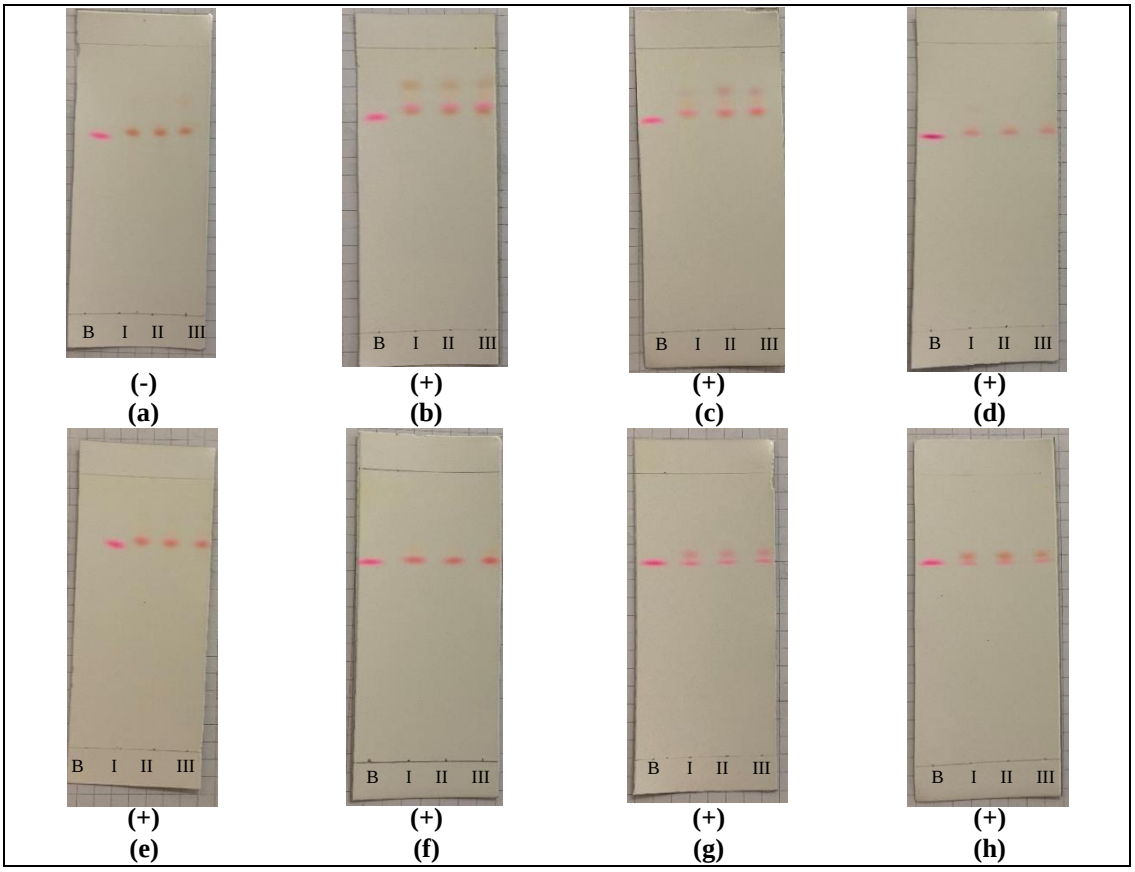

Figure 2. Visually spot sample

Information : (a) Sample negative
(b) Sample A3
(c) Sample A4
(d) Sample A6
B : Standard
I : Replication 1
(e) Sample A7
(f) Sample A8
(g) Sample A11
(h) Sample A12
II : Replikasi 2
III : Replikasi 3

Figure 2. shows the sample stain visually based on the shape and color, a positive result of a sample containing Rhodamine B is indicated by a bright pink stain and a round/elliptical stain in Figure (b); (c); (d); (e); (f); (g) and (h).

The positive and negative results on samples containing Rhodamine B at 245 nm UV light can be seenin Figure 3.

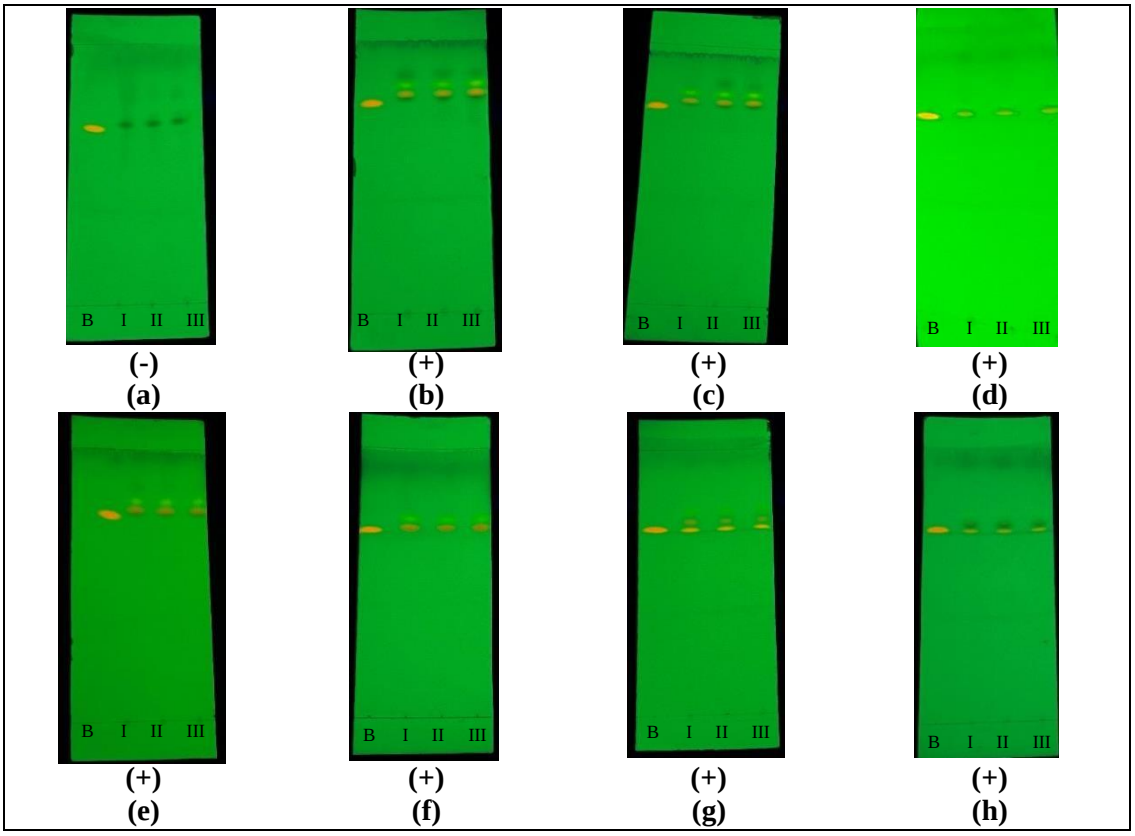

Figure 3. Sample spot under 254 nm UV light

Information : (a) Sample negative
(b) Sample A3
(c) Sample A4
(d) Sample A6
B : Standard
I : Replication 1
(e) Sample A7
(f) Sample A8
(g) Sample A11
(h) Sample A12
II : Replikasi 2
III : Replikasi 3

Figure 3. shows sample stain under 254 nm UV light based on the shape and color of the stain, a positive result of a blush sample containing Rhodamine B is indicated by a yellow or orange fluorescent stain and a round/elliptical shape of the stain (b); (c); (d); (e); (f); (g) and (h), while the stains on the blush sample that did not contain Rhodamine B were shown by stains that did not fluoresce yellow or orange in Figure (a).

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

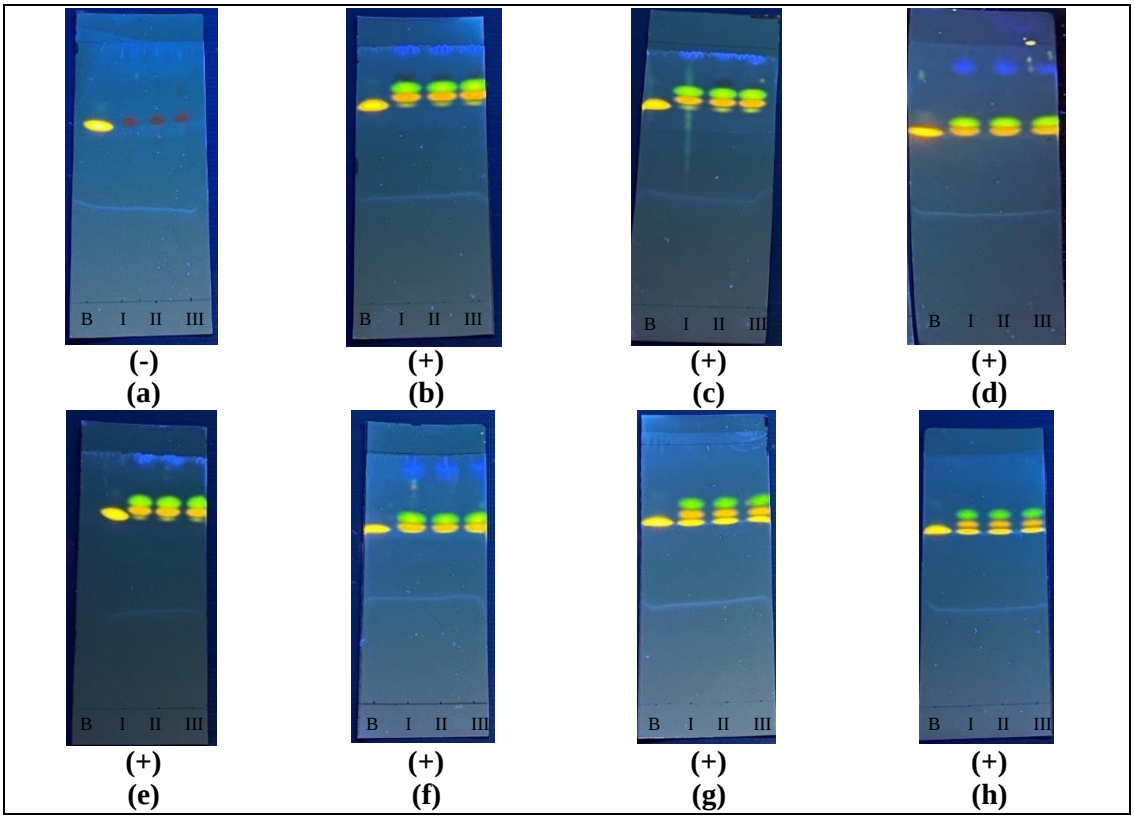

Figure 4. Sample spot under 366 nm UV light

Information : (a) Sample negative
(b) Sample A3
(c) Sample A4
(d) Sample A6
B : Standard
I : Replication 1
(e) Sample A7
(f) Sample A8
(g) Sample A11
(h) Sample A12
II : Replikasi 2
III : Replikasi 3

Figure 4. shows stain under 366 nm UV light based on the shape and color of the stain, positive results of samples containing Rhodamine B are indicated by yellow or orange fluorescent stains and round/elliptical stains in Figure (b); (c); (d); (e); (f); (g) and (h).

DISCUSSION

Identification of Rhodamine B dye in cosmetic blush on sold in Marketplace X can be done using the TLC (Thin Layer Chromatography) test method, the TLC method is a method that can detect Rhodamine B compounds because Rhodamine B has chromophore and auxochrome groups so that Rhodamine B can fluoresce if observed under UV 254 nm and UV 366 nm. The standard solution is made by dissolving Rhodamine B powder in methanol as a position control and a solution is made by dripping 4 M HCl as much as 4 drops which serves to destroy the compound in the blush on sample and the Rhodamine B compound in the blush on sample so that it does not ionize into a neutral form (Yuniarto et al., 2019) which was then dissolved by methanol and filtered to separate the insoluble particles. Rhodamine B hasthe property of being very soluble in organic solvents such as methanol so that methanol is used to dissolveRhodamine B because methanol is polar so it can dissolve organic substances as well as polar (Sari et al., 2022).

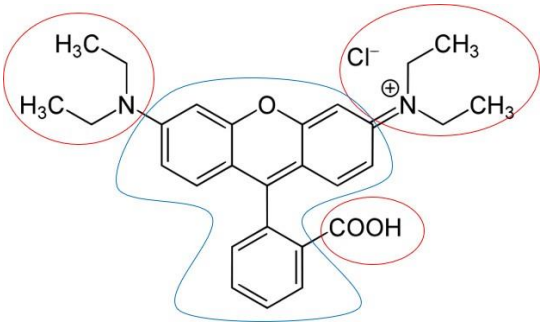

Figure 5. Chromophore and Auxochrome groups of Rhodamine B Compounds

——— = Chromophore group

——— = Auxochrome group

Rhodamine B has auxochrome and chromophore groups which can be seen in Figure 5. Rhodamine B has chromophore groups that are able to absorb UV and visible light in organic compounds, namely carboxyl groups and aromatic compounds. Rhodamine B also has an auxochrome group, which is a group that has a free electron pair such as NR2 (Fauziah et al., 2020). In this study, silica gel TLC plate type Gf254 was used, which means that the silica gel can fluoresce at UV 254 nm (Indrayani et al., 2017). silica gel contains silanol (Si-OH) and siloxane (Si-O-Si) groups on its surface where the silanol groups can interact with the analyte because it forms hydrogen interactions. The silanol group can interact with water so that the TLC plate (silica gel) can be deactivated, so it is necessary to heat it in the oven to dry the water molecules on the plate so that the TLC plate becomes active (Rosamih, 2019). On the surface of the silica gel can be reactivated and the silanol group can interact with the analyte (Ediningtyas, 2012)

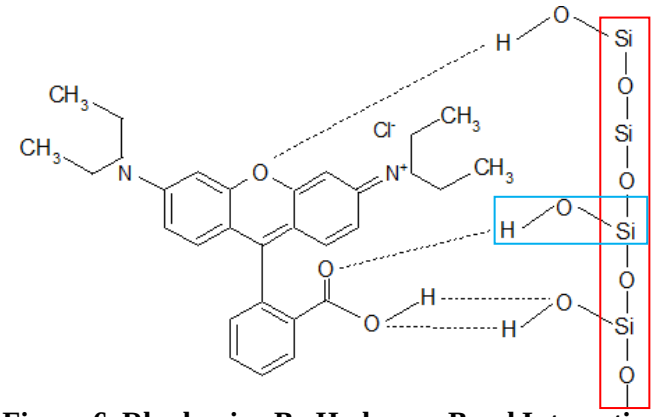

Figure 6. Rhodamine B . Hydrogen Bond Interaction

..... = Hydrpgen interaction

——— = Siloxane group

——— = Silanol group

The interaction between silica gel and Rhodamine B compounds in Figure 6. will form hydrogen bonds in which Rhodamine B is polar and the silica gel is also polar which will cause strong adsorption so that the Rhodamine B analyte compound will be retained on the silica gel and the analyte on the silica gel will propagate longer (Ediningtyas, 2012). Rhodamine B is polar because its molecular structure has a carboxyl group with a lone pair of electrons so that the choice of eluent needs to be adjusted. Rhodamine B has a carboxyl and amine group that will form intermolecular hydrogen bonds with polar solvents so that Rhodamine B compounds are easily soluble and can produce good separation between Rhodamine B compounds and other compounds (Nafiq et al., 2020). In this study, the chamber that has been filled with the mobile phase needs to be saturated. The saturation function is to ensure that the particles from the mobile phase will be evenly distributed throughout the chamber so that the spots in the stationary phase will move optimally by the mobile phase, which means that the saturation is to optimize the rise of the eluent (Samosir et al., 2018). Optimization of the mobile phase was carried out to find a suitable mobile phase for the separation of Rhodamine B compounds contained in blush on cosmetic so as to obtain optimal separation, namely to produce round/elliptical and tailless spots (Elfasyari et al., 2020). The

sample is declared positive if it enters the sample stain parameter criteria that are parallel to the Rhodamine B standard which is visually bright pink if the distance between the Rf value of the stain between the standard and the sample is the same or close to each other with a price difference of 0.2 (Fauziah et al., 2020), fluoresces orange under UV light of 254 nm and 366 nm (Hiola et al., 2021) the Rf value enters the range of 0.2-0.8 (Elfasyari et al., 2020) and there is a good separation between the 2 stains if the value of Rs 1.5 (Harum, 2021).

CONCLUSION

Based on the results of the research on Rhodamine B content analysis by optimizing the Mobile Phase Using the Thin Layer Chromatography Method in Blush On Cosmetics sold on Marketplace X, it can be concluded that: Spots with positive results are visually round/elliptical and light pink in color according to the color of the standard Rhodamine B stain. Observations of spot under UV light of 254 nm and 366 nm fluoresce in yellow or orange. The optimal separation of Rhodamine compounds is the mobile phase mixture of n-Butanol: Ethanol 96%: Aquadest (20:12:15). 12 of blush samples studied, there were 7 samples containing Rhodamine B with an Rf value of 0.79 for sample A3; sample A4 0.77; sample A6 0.68; sample A7 0.76; sample A8 0.69; sample A11 0.68 and sample A12 Rf value of 0.69.

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