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Detection and Quantification of Rhodamine B in Loose Powder and Blush Cosmetic Products

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ABSTRACT

The use of decorative cosmetics such as loose powder and blush is increasingly widespread, raising concerns regarding the safety of the coloring agents used, particularly Rhodamine B. This study aimed to identify the presence and determine the levels of Rhodamine B in loose powder and blush cosmetic products using Thin Layer Chromatography (TLC) for qualitative analysis and High-Performance Liquid Chromatography (HPLC) for quantitative analysis. The HPLC method applied in this study was validated and showed good linearity, precision, and sensitivity in accordance with ICH guidelines. A total of 16 cosmetic samples were analyzed, and 3 samples were confirmed to contain Rhodamine B. The highest Rhodamine B level was found in sample X at 66.54 µg/g, followed by sample Z at 21.03 µg/g, and sample Y at 0.83 µg/g. These findings indicate that the presence of Rhodamine B in cosmetic products remains a concern. Therefore, it is necessary to strengthen regulatory supervision and increase public awareness to select safe cosmetic products that are officially registered by BPOM.

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ABSTRAK

Penggunaan kosmetik dekoratif seperti bedak tabur dan perona pipi semakin meluas, sehingga menimbulkan kekhawatiran terkait keamanan bahan pewarna yang digunakan, terutama Rhodamin B. Penelitian ini bertujuan untuk mengetahui keberadaan dan kadar Rhodamin B pada produk kosmetik bedak tabur dan perona pipi menggunakan Kromatografi Lapis Tipis (KLT) untuk analisis kualitatif dan Kromatografi Cair Kinerja Tinggi (KCKT) untuk analisis kuantitatif. Metode KCKT yang digunakan telah divalidasi dan menunjukkan linearitas, presisi, serta sensitivitas yang baik sesuai pedoman ICH. Sebanyak 16 sampel kosmetik dianalisis, dan 3 sampel di antaranya terdeteksi positif mengandung Rhodamin B. Kadar Rhodamin B tertinggi ditemukan pada sampel X sebesar 66,54 µg/g, diikuti sampel Z sebesar 21,03 µg/g, dan sampel Y sebesar 0,83 µg/g. Temuan ini menunjukkan masih adanya risiko kontaminasi Rhodamin B pada produk kosmetik di pasaran. Oleh karena itu, diperlukan pengawasan yang lebih ketat dari pihak regulator serta peningkatan kesadaran konsumen untuk memilih produk kosmetik yang aman dan telah terdaftar resmi di BPOM.

Kata Kunci: Rhodamin B; Kosmetik; Kromatografi Lapis Tipis; Kromatografi Cair Kinerja Tinggi; Keamanan Kosmetik.

1. Introduction

The increasing use of decorative cosmetics, such as blush, eyeshadow, and loose powder, not only among women but also among men, has raised concerns regarding the safety of cosmetic ingredients [1], [2]. One of the main concerns is the illegal use of synthetic dyes such as Rhodamine B, a xanthene dye widely utilized in the textile, paint, and paper industries, but strictly prohibited in cosmetic products due to its toxic and carcinogenic properties [3], [4], [5].

Rhodamine B exposure may cause respiratory tract irritation, promote cancer development, and lead to liver damage when accumulated in the body at high concentrations [6], [7], [8]. The Indonesian Food and Drug Authority (BPOM), through Regulation No. 18 of 2015, has prohibited the use of several hazardous substances in cosmetics, including mercury, retinoic acid, hydroquinone, and synthetic dyes such as Rhodamine B (Red K3 and Red K10) [9].

However, despite the existing regulation, the circulation of harmful cosmetics remains a public health issue. The latest BPOM report (November 2023 to October 2024) revealed that 55 cosmetic products sold in Indonesia were found to contain prohibited and hazardous substances, including Rhodamine B, highlighting the persistence of this problem, especially in unregistered products marketed online and in traditional outlets [10].

Previous studies have identified Rhodamine B in cosmetic products using High-Performance Liquid Chromatography (HPLC) analysis [11], [12]. Meanwhile, Thin Layer Chromatography (TLC) is widely employed due to its simplicity and effectiveness in the qualitative identification of various compounds, including Rhodamine B [13]. Nevertheless, studies combining both TLC and HPLC for the simultaneous identification and quantification of Rhodamine B in cosmetic products are still limited [14].

Therefore, this study aimed to identify the presence and determine the levels of Rhodamine B in loose powder and blush products using TLC for qualitative analysis and HPLC for quantitative analysis, in order to provide scientific data to support cosmetic safety monitoring in Indonesia.

2. Methods

Research Design Sample Collection

This study was a descriptive laboratory study designed to identify the presence and determine the levels of Rhodamine B in loose powder and blush cosmetic products. Qualitative analysis was performed using Thin Layer Chromatography (TLC), while quantitative analysis was conducted using High-Performance Liquid Chromatography (HPLC).

Sample Collection

The samples analyzed consisted of sixteen cosmetic products, including four loose powders (one unregistered and three BPOM-registered), six blushes (three unregistered and three BPOM-registered), and six eyeshadows (three unregistered and three BPOM-registered). These samples were collected from online marketplaces and traditional markets with a high circulation of cosmetic products.

Materials

The materials used in this study included Rhodamine B standard (Sigma-Aldrich), analytical grade methanol, acetonitrile, ethyl acetate, n-butanol, 25% ammonia solution, and distilled water. Cosmetic samples tested were in the form of loose powder, blush, and eyeshadow, consisting of both registered and unregistered products.

Instruments

The instruments used included an HPLC system (Rigol L-3000) with a UV-Vis detector, a reversed-phase C18 column (4.6 mm × 250 mm, 5 µm), TLC plates coated with Silica Gel GF254 (20 cm × 20 cm), a UV lamp at 254 nm, an ultrasonic vibrator, an analytical balance (±0.0001 g), membrane filters (Millipore 0.45 µm), capillary tubes for TLC spotting, and standard laboratory glassware.

Qualitative Analysis Procedure Using Thin Layer Chromatography (TLC)

The TLC analysis referred to the method by Rahman et al. (2023) [15] with slight modifications. A total of 500 mg of each cosmetic sample was weighed and dissolved in 5 mL of analytical grade methanol. The Rhodamine B standard solution was prepared by dissolving 50 mg of the dye in 50 mL of methanol. The mobile phase used consisted of ethyl acetate, n-butanol, and 25% ammonia in a 20:55:25 (v/v/v) ratio.

Samples and standard solutions were applied to TLC plates using a capillary tube at 1 cm from the bottom. The plates were developed in a saturated chromatography chamber, then removed, dried, and observed under a UV lamp at 254 nm. The presence of Rhodamine B was confirmed by the appearance of pink spots under visible light and yellow or orange fluorescence under UV light. The Rf value was calculated and compared to the Rhodamine B standard [16].

Quantitative Analysis Procedure Using High-Performance Liquid Chromatography (HPLC)

The quantitative analysis referred to the method used by Rachmawati et al. (2017) [13] with several modifications. The mobile phase consisted of acetonitrile, methanol,

and distilled water in a 47:47:6 (v/v/v) ratio. This solution was homogenized using an ultrasonic vibrator for 15 minutes and filtered through a 0.45 µm membrane filter.

The HPLC system was operated using a reversed-phase C18 column (4.6 mm × 250 mm, 5 µm), with a detection wavelength of 554 nm, a flow rate of 1 mL/min, an injection volume of 20 µL, and ambient room temperature.

A 1000 ppm stock solution of Rhodamine B was prepared by dissolving 50 mg in 50 mL of methanol. From this, a 100 ppm working solution was prepared, and serial dilutions were made to obtain standard concentrations of 2, 4, 6, 8, 10, and 12 ppm. Cosmetic samples were prepared by dissolving 500 mg of each product in 5 mL of methanol, homogenized, filtered through a 0.45 µm membrane, and injected into the HPLC system [17], [18].

Method Validation Parameters

Method validation was conducted to ensure the accuracy and reliability of the HPLC method used for Rhodamine B quantification. The validation procedure followed the guidelines of the International Conference on Harmonization (ICH) [19] and previous studies by Komarudin et al. (2019) [17].

The validated parameters included linearity, limit of detection (LOD), limit of quantification (LOQ), and precision. Linearity was evaluated by preparing standard Rhodamine B solutions at concentrations ranging from 2 to 12 ppm and plotting the peak area against concentration. Linearity is considered acceptable if the correlation coefficient (r^2) is greater than 0.99, indicating a strong relationship between concentration and response [19], [20].

Precision was evaluated by repeated analysis of each cosmetic sample (n=6), and expressed as the relative standard deviation (%RSD), which was calculated using the formula:

$$\%RSD = \frac{Standard\ Deviation}{Mean} \times 100\%$$

The limit of detection (LOD) and limit of quantification (LOQ) were determined based on the International Conference on Harmonization (ICH) guidelines [19], [20]. The formulas used are:

$$LOD = 3.3 \times \frac{SD}{Slope}$$

$$LOQ = 10 \times \frac{SD}{Slope}$$

Where SD is the standard deviation of the response, and Slope is the slope obtained from the calibration curve of Rhodamine B.

3. Results and Discussion

Thin Layer Chromatography (TLC) Analysis

Thin Layer Chromatography (TLC) was performed to identify the presence of Rhodamine B in cosmetic samples. This method involves two phases, namely the stationary phase and the mobile phase. The stationary phase used in this analysis was silica gel GF254, while the mobile phase was a mixture of ethyl acetate, n-butanol, and 25% ammonia in a ratio of 20:55:25 (v/v/v). This mobile phase provides effective separation and adequate selectivity for Rhodamine B from other components present in the sample matrix [15].

Samples and Rhodamine B standard were spotted onto the TLC plate using a capillary tube, then placed in the chromatography chamber containing the mobile phase. The mobile phase migrated upward through capillary action. After the development was completed, the plate was dried and observed under visible light and UV light at 254 nm.

The presence of Rhodamine B was indicated by a pink spot under visible light and yellow-orange fluorescence under UV light. The Rf (retardation factor) value was calculated as the ratio of the distance traveled by the compound to the distance traveled by the solvent front.

Table 1. Qualitative Analysis Results Using Thin Layer Chromatography

Sample Code	Observation		Rf Value	Conclusion
	Visual	UV light 254 nm		
Rhodamine B	Pink	Fluoresces yellow	0.487	Comparator Standard
Code 1	Purple	No Fluorescence	-	Negative
Code 2	Purple	No Fluorescence	-	Negative
Code 3	Pink	Fluoresces yellow	0.481	Positive
Code 4	Pink	Fluoresces yellow	0.487	Positive
Code 5	Purple	No Fluorescence	-	Negative
Code 6	Pink	Fluoresces yellow	0.475	Positive
Code 7	Purple	No Fluorescence	-	Negative
Code 8	Purple	No Fluorescence	-	Negative
Code 9	Purple	No Fluorescence	-	Negative
Code 10	Purple	No Fluorescence	-	Negative
Code 11	Purple	No Fluorescence	-	Negative
Code 12	Purple	No Fluorescence	-	Negative
Code 13	Purple	No Fluorescence	-	Negative
Code 14	Purple	No Fluorescence	-	Negative
Code 15	Purple	No Fluorescence	-	Negative
Code 16	Purple	No Fluorescence	-	Negative

Description: *Code 3 = Sample Z*
Code 4 = Sample X
Code 6 = Sample Y

The results of the TLC analysis are presented in **Table 1**. Samples that exhibited fluorescence and Rf values close to the Rhodamine B standard (Rf = 0.487) were identified as positive. Three samples (Code 3, Code 4, and Code 6) showed Rf values of 0.481, 0.487, and 0.475, respectively, which confirmed the presence of Rhodamine B.

Meanwhile, the other samples (Code 1, 2, 5, and Codes 7 to 16) were identified as negative for Rhodamine B because they did not exhibit yellow-orange fluorescence and had Rf values that differed significantly from the standard. These samples are most likely categorized as loose powders or eye products, such as eyeshadow, which rarely utilize Rhodamine B as a colouring agent due to different formulation characteristics [21].

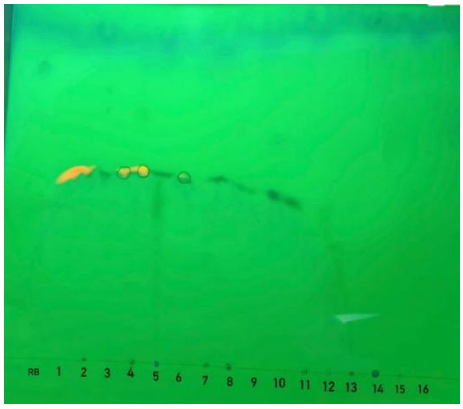

Figure 1. TLC results under UV light at 254 nm showing yellow-orange fluorescence in samples Code 3, Code 4, and Code 6, indicating the presence of Rhodamine B.

The visual observation of TLC results under UV light can be seen in **Figure 1**, showing fluorescent spots for Code 3, Code 4, and Code 6, while other samples showed no fluorescence. These results are consistent with Rachmawati et al. (2017) [13], who reported the effectiveness of TLC in detecting Rhodamine B in blush products based on fluorescence and Rf values.

Based on this analysis, it can be concluded that only Code 3 (Sample Z), Code 4 (Sample X), and Code 6 (Sample Y) were confirmed to contain Rhodamine B. The detection of this prohibited compound highlights the importance of strict monitoring, especially for unregistered cosmetic products sold in the market, in accordance with BPOM Regulation No. 18 of 2015 [9].

High Performance Liquid Chromatography (HPLC) Analysis

Method Validation

The validation of the HPLC method in this study was carried out to ensure the accuracy, precision, and sensitivity of Rhodamine B determination in cosmetic samples. The validated parameters included linearity, limit of detection (LOD), limit of quantification (LOQ), and precision.

Linearity was evaluated using six concentrations of Rhodamine B standard solutions (2, 4, 6, 8, 10, and 12 ppm). The regression equation obtained was $y = 231.081x + 25.3762$, with a correlation coefficient (r^2) of 0.9986, meeting ICH requirements for good linearity ($r^2 \geq 0.99$) [19]. The calibration curve results are shown in **Table 2**.

Table 2. Rhodamine B calibration curve

No.	Used	Response (mAU.S)	Amount (mg/mL)	Response Factor	Recalibration Level
1	√	453.83	2.00	0.00	0
2	√	1028.54	4.00	0.00	0
3	√	1365.09	6.00	0.00	0
4	√	1859.69	8.00	0.00	0
5	√	2358.34	10.00	0.00	0
6	√	2792.17	12.00	0.00	0

LOD and LOQ were calculated based on the formula recommended by the International Conference on Harmonization (ICH) guidelines, where LOD = 3.3 × (SD/Slope) and LOQ = 10 × (SD/Slope) [19]. In this formula, SD represents the standard deviation of the response from the calibration curve, and Slope refers to the slope value obtained from the linear regression of Rhodamine B standard concentrations.

The calculated results revealed that LOD and LOQ obtained were 0.21 ppm and 0.63 ppm, respectively. The LOQ value obtained in this study was 0.63 ppm, indicating the minimum concentration of Rhodamine B that can be quantitatively determined with acceptable accuracy and precision [19]. This result aligns with previous studies that reported LOQ values for Rhodamine B in the range of 0.3737 to 0.5617 mg/kg using UV-Vis spectrophotometry in food samples [22]. Additionally, Tatebe et al. (2014) reported LOQ values between 0.025 and 0.125 µg/g for Rhodamine B in various processed foods using HPLC methods. These findings support the sensitivity and reliability of the HPLC method employed in this study for detecting low concentrations of Rhodamine B in cosmetic samples [23].

These findings confirm that the HPLC method developed in this study had good sensitivity and was capable of detecting and quantifying Rhodamine B in cosmetic samples even at very low concentrations. This sensitivity is essential, considering that Rhodamine B is a prohibited dye in cosmetic products, and its presence, even in trace amounts, must be identified and controlled to prevent potential health risks to consumers [9].

Precision testing was evaluated by repeated analysis (n=6) for each cosmetic sample group, and the results were expressed as the relative standard deviation (%RSD). The detailed %RSD results for each sample are presented in **Table 3**, indicating good repeatability as all values were within the acceptance criteria (%RSD ≤ 5%) [19]. The %RSD values obtained were 0.16% for sample X, 2.26% for sample Y, and 0.20% for sample Z. These values indicate good precision and acceptable repeatability for all samples, as they fall within the acceptance criteria of %RSD ≤ 5% for assay methods, as recommended by ICH Q2(R1) guidelines [19].

The low %RSD value in sample X (0.16%) indicates excellent consistency of the results, reflecting a stable and homogeneous sample matrix. Similarly, sample Z showed low variability (%RSD 0.20%). Meanwhile, the highest %RSD value was observed in sample Y (2.26%), which, although higher than the other groups, still meets the acceptable limit and suggests slight variability, possibly due to the lower concentration of Rhodamine B in this group, which may affect measurement stability.

These findings are also in accordance with AOAC guidelines, which state that acceptable %RSD values for repeatability depend on the analyte concentration, with higher %RSDs permissible at lower concentrations [20].

Quantitative Analysis Results of Rhodamine B

Quantitative analysis of Rhodamine B content in cosmetic samples was carried out using HPLC with a C18 reversed-phase column, and the mobile phase consisted of acetonitrile, methanol, and aquabidest (47:47:6 v/v/v). The flow rate was set at 1 mL/min, and detection was performed at a wavelength of 554 nm.

The results of Rhodamine B levels in the cosmetic samples are shown in **Table 3**. The results indicated that group X had the highest Rhodamine B concentration (66.5426 µg/g), group Z had a moderate level (21.0286 µg/g), and group Y had the lowest concentration (0.8258 µg/g).

Table 3. Quantitative Test Data Analysis Results

Sample Name		Level (µg/g)	Average Level (µg/g)	Standard Deviation (µg/g)	%RSD
X	X1	66.2079	66.5426	0.1095	0.16%
	X2	66.7171			
	X3	66.4512			
	X4	66.6930			
	X5	66.5490			
	X6	66.6370			
Y	Y1	0.8042	0.8258	0.0187	2.26%
	Y2	0.8537			
	Y3	0.8360			
	Y4	0.8286			
	Y5	0.8060			
	Y6	0.8261			
Z	Z1	21.0141	21.0286	0.0424	0.20%
	Z2	20.9989			
	Z3	21.0847			
	Z4	20.9116			
	Z5	21.0716			
	Z6	21.0908			

The analysis results showed that the Rhodamine B level in group X was the highest, which may be due to the type of product suspected to be blush, requiring a higher intensity of color. The %RSD of 0.16% indicated a high consistency of results.
Group Z had a Rhodamine B level of 21.0286 µg/g, lower than group X but higher than group Y, possibly due to differences in the type of cosmetic or formulation. Group Y showed the lowest Rhodamine B level (0.8258 µg/g), suspected to be loose powder or eyeshadow, which typically uses a minimal amount of colorant [24].
These findings align with the results of Puspitasari et al. (2023) [24], which also found significant variations in Rhodamine B levels in cosmetics based on product type and production quality control.

According to BPOM Regulation No. 18 of 2015 [9], Rhodamine B is strictly prohibited in cosmetic formulations because it poses serious health risks, such as respiratory tract irritation, liver toxicity, and carcinogenic effects [3], [6]. The presence of Rhodamine B in these samples, especially at high levels in sample X, is concerning and indicates the need for strict regulation and public awareness. Consumers are advised to purchase cosmetic products from official or BPOM-registered stores to minimize the risk of exposure to hazardous substances like Rhodamine B.

This study has several limitations. The accuracy parameter (recovery test) was not conducted due to the absence of standard addition procedures. Additionally, the stability of Rhodamine B in the cosmetic matrix over time was not evaluated. Further studies are recommended to include accuracy and stability tests, as well as broader sample types, to provide a more comprehensive safety assessment of Rhodamine B contamination in cosmetics.

4. Conclusion

This study confirmed that out of 16 cosmetic samples analyzed, three samples consisting of loose powder and blush products were found to contain Rhodamine B. The highest concentration was found in sample X, followed by sample Z and sample Y. The

presence of Rhodamine B in these cosmetic products raises serious health concerns due to its toxic and carcinogenic properties. These findings highlight the importance of strict regulatory control and supervision of cosmetic products circulating in the market to protect consumers from the potential health risks associated with the use of hazardous substances.

Based on the results of this study, it is recommended that regulatory agencies, particularly BPOM, increase routine supervision and monitoring of cosmetic products, especially those distributed through online platforms and traditional markets. Cosmetic manufacturers are also expected to comply with applicable regulations by avoiding the use of prohibited substances such as Rhodamine B. Consumers are advised to be more selective by purchasing only officially registered and BPOM-certified cosmetic products, thereby minimizing the risk of exposure to hazardous substances. Furthermore, future research is suggested to include accuracy (recovery) testing, stability studies, and a wider variety of cosmetic samples to provide a more comprehensive evaluation of Rhodamine B contamination in cosmetics.

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Conflicts of Interest:
The authors declare no conflicts of interest.

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