Jurnal Ilmiah Farmasi (Scientific Journal of Pharmacy) 21(2) June-September 2025, 193-205
ISSN: 1693-8666
available at http://journal.uii.ac.id/index.php/JIF

The analysis of hydroquinone levels in the product of whitening hand and body lotion in online shops, using the UV-Vis spectrophotometry method

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Abstract

Background: Hydroquinone serves as a skin-lightening agent by suppressing melanin production. However, its usage in cosmetic formulations has been prohibited under the Indonesian Food and Drug Authority regulations, as stated in Regulation Number 18 of 2015. Despite this restriction, numerous studies show that various whitening hand and body lotion products commonly contain hydroquinone.
Objective: This study aimed to conduct a qualitative and quantitative analysis of hydroquinone in body-whitening hand and body lotion products available through online retail platforms.
Method: The methodology employed in this study involved the use of Thin Layer Chromatography (TLC) for qualitative analysis, complemented by UV-Vis spectrophotometry, which had undergone prior validation to serve as a quantitative analytical tool.
Results: The qualitative analysis conducted via Thin Layer Chromatography (TLC) identified six positive samples containing hydroquinone, characterized by an Rf value of 0.75. According to the validation results, the method demonstrated a linearity value with a correlation coefficient (r) of 0.9976, a limit of detection (LOD) of 0.3862 g/mL, and a limit of quantification (LOQ) of 1.2872 g/mL. An RSD value of 1.912% showed precision, while accuracy was confirmed through a recovery rate of 96.77%. The validation outcomes suggested that this approach is robust for analyzing hydroquinone in body-whitening hand and body lotion products. The UV-Vis spectrophotometry result showed that samples 1 through 6 had hydroquinone in them at levels of 0.0418, 0.0448, 0.0361, 0.0283, 0.0365, and 0.3193%, respectively.
Conclusion: The research conducted revealed that the qualitative analysis using Thin-Layer Chromatography (TLC) yielded six positive samples, indicating the presence of hydroquinone. Furthermore, the quantitative analysis indicated that whitening hand and body lotion products available in online shops contained hydroquinone at concentrations ranging from 0.0283 to 0.3193 g/mL. This finding underscores the continued prevalence of prohibited chemicals in certain cosmetic products.
Keywords: hand body lotion, hydroquinone, online shop, UV-Vis spectrophotometry, method validation

1. Introduction

Skincare has evolved into both a trend and an essential aspect of self-care for modern women. Among the widely utilized cosmetic preparations, hand and body lotions that whiten the skin remain particularly popular among women in general (Sari *et al.*, 2021). With the evolution of the economy and the globalization wave, shopping habits within society have undergone significant transformations. Traditionally, sales transactions were conducted through direct meetings between buyers and sellers. However, the rapid advancements in technology have shifted most of these interactions to online platforms. The rise of e-commerce and online media presents a substantial opportunity for cosmetic traders and entrepreneurs to market their products widely. Moreover, the prospect of acquiring goods at more affordable prices is increasingly attracting many consumers (Sari, 2015).

Cosmetic products have emerged as a lucrative sector within trade due to their capacity to generate substantial revenue. But many manufacturers don't follow product quality standards because they care more about making money than about the health of their customers. This disregard has led to the widespread distribution of cos metic products in the market that contain substances failing to meet established safety and eligibility requirements for use (Suhartini & Fatimawali, 2013).

Since cosmetics are used continuously and routinely by individuals, ensuring their safety is imperative, particularly regarding whether their active ingredients may provoke adverse reactions or pose risks to skin and overall health. In Indonesia, people use cosmetics for both short-term and long-term purposes, making this especially crucial. Dermatologists frequently encounter incidents of side effects from cosmetic usage. The inclusion of potentially addictive substances designed to intensify whitening effects often causes severe adverse reactions (Lisnawati & Wijayanti, 2016).

Hydroquinone is widely utilized in the treatment of pigmentation disorders due to its pharmacological ability to act as a competitive inhibitor of tyrosinase, a key enzyme involved in the biosynthesis of melanin. By interfering with this enzymatic activity, hydroquinone effectively reduces melanin production, making it a valuable agent in managing conditions such as melasma, hyperpigmentation, and other skin discolorations (Searle *et al*., 2021). Hydroquinone is among the most extensively utilized and recognized ingredients for skin whitening. Chemically, it is represented by white, needle-shaped crystals that are odorless and have the formula $C_6H_4(OH)_2$. It is also known by the chemical name 1,4-benzenediol or quinol. This compound functions as a whitening agent through a mechanism that inhibits pigmentation by suppressing the activity of the tyrosinase enzyme, which plays a critical role in the process of skin darkening (Gianti, 2013). Based on the findings of Banodkar and Banodkar (2022), hydroquinone is generally deemed safe for use in managing pigmentation disorders, provided it is administered at the recommended concentration levels and under strict medical supervision. Their review emphasizes the necessity of adhering to proper dosage guidelines to mitigate potential risks of toxicity and long-term side effects, highlighting that misuse or overuse can lead to adverse outcomes such as ochronosis or skin irritation. This reinforces the importance of controlled use, particularly in prescription settings, to optimize safety and efficacy.

Drawing from previous research conducted by Faisal *et al.* (2018) and Sari *et al.* (2021) on the detection of hydroquinone in whitening hand and body lotions, despite its prohibition due to associated health risks, we sought to examine the content and concentration of hydroquinone in cosmetic hand and body lotion formulations. Therefore, this study aimed to analyze the presence of hydroquinone and quantify the concentrations in products distributed and sold through online platforms as part of efforts to

mitigate health risks associated with cosmetic use. The qualitative analysis in this study was carried out using Thin Layer Chromatography (TLC), selected for its simplicity in separating analyte mixtures through a two-phase system. Meanwhile, the quantitative analysis employed UV-Vis spectrophotometry, as hydroquinone contains a chromophore group suitable for detection and measurement using this method (Faisal *et al*., 2018; Sari *et al*., 2021).

2. Method

2.1. Sample collections and organoleptic test

Six samples were selected through a purposive sampling method. All samples were procured from two distinct online shops, chosen based on their categorization as best-selling whitening hand and body lotion products. The selected products did not disclose their composition, nor did a BPOM registration number accompany them. The body-whitening hand and body lotion products were characterized based on their respective shape, color, and scent.

2.2. Qualitative test

2.2.1 Sample preparation for TLC

A one-gram sample was dissolved in approximately 5 mL of 96% ethanol and transferred to a 25 mL volumetric flask, after which additional 96% ethanol was added up to the calibration mark and the solution was thoroughly mixed until homogeneous before being filtered (Simaremare, 2019). The stationary phase utilized in the procedure was silica gel GF_{254}.

2.2.2 Qualitative analysis with TLC

An eluent solution was prepared by mixing toluene and glacial acetic acid in a 2:8 ratio, resulting in a total volume of 30 mL. This solution was then poured into the chamber and allowed to saturate for 30 minutes. Samples 1 through 6 and a hydroquinone standard were applied to the TLC plate using a 1 µL capillary tube before being placed into the chamber containing the eluent. The solvent was allowed to ascend the plate until it reached the designated line, at which point the plate was removed from the chamber. The spots were subsequently observed under a 254 nm UV light, and their positions were measured (Simaremare, 2019).

2.3. Quantitative analysis preparations

2.3.1 Preparation of hydroquinone standard solution

A total of 10 mg of hydroquinone was measured and dissolved in 2 mL of methanol. This solution was then transferred into a 100 mL volumetric flask, to which methanol was added until the volume reached 100 mL, followed by thorough shaking to ensure homogeneity, thereby yielding a hydroquinone

standard concentration of 100 ppm. From this 100 ppm solution, 25 mL was extracted and further diluted with 50 mL of methanol, with the mixture being shaken until homogeneous, resulting in a standard hydroquinone concentration of 50 ppm (Adriani & Safira, 2019).

2.3.2 Determination of wavelength

The absorbance of a hydroquinone standard solution at a concentration of 14 ppm was measured to determine its maximum wavelength using UV-Vis spectrophotometry within a wavelength range of 200-400 nm (Adriani & Safira, 2019).

2.3.3 Determination of operating time (OT)

The absorbance of a 14 ppm hydroquinone standard solution was measured at its maximum wavelength until a relatively stable absorbance value was achieved. Readings were taken at intervals of every five minutes over a total period of 30 minutes (Adriani & Safira, 2019).

2. 4. Method validation

2.4.1 Linearity

Standard solutions at concentrations of 10, 14, 18, 22, and 26 ppm were analyzed using a UV-Vis spectrophotometer, employing the maximum wavelength identified in prior wavelength determinations, with methanol serving as the blank. The linearity of the data was statistically evaluated to determine the correlation coefficient (r), slope, and regression constant (Irnawati *et al*., 2016; Adriani & Safira, 2019).

2.4.2 Precision

The absorbance of a hydroquinone standard solution with a concentration of 26 ppm was measured at the maximum wavelength six times. The resulting data were then used to determine the Standard Deviation (SD) and Relative Standard Deviation (RSD) values (Irnawati *et al*., 2016).

2.4.3 Accuracy

A positive sample containing hydroquinone was accurately weighed to 25 mg and then dissolved in 20 mL of methanol. Employing the standard addition method, hydroquinone standard solutions at concentrations of 10, 18, and 26 ppm were incorporated into the sample solution. The absorbance of each resultant mixture was subsequently measured at the maximum wavelength using a UV-Vis spectrophotometer. The values obtained were used to calculate the % recovery (Irnawati *et al*., 2016).

2.4.4 LOD and LOQ

The limit of detection (LOD) and the limit of quantification (LOQ) are key parameters characterizing the performance of the whole test method at low concentrations (European Union, 2016). The limit of detection (LOD) and limit of quantification (LOQ) were determined statistically using the linear regression equation derived from the standard calibration curve.

2.5. Determination of hydroquinone levels

Each sample was weighed to 100 mg and dissolved in 50 mL of methanol, followed by thorough mixing to ensure homogeneity. The resulting solution was then filtered using filter paper and transferred into a cuvette for individual measurement with a UV-Vis spectrophotometer, employing the previously determined maximum wavelength (Adriani & Safira, 2019).

3. Result and discussion

3.1. Sample organoleptic test

Four of the six samples analyzed (sample codes 2, 3, 4, and 5) emitted a perfume-like scent accompanied by a sharp, medicinal odor. In contrast, the remaining two samples exhibited a mild and pleasant fragrance resembling that of perfume without being overpowering. The strong, pungent aroma observed in certain hand and body lotion products may indicate the presence of harmful substances, as such scents are often employed to mask the odors associated with potentially hazardous additives in the formulation (Mohamad, 2014).

The organoleptic assessment revealed a range of colors in four out of the six samples. This variation in the coloring of hand and body lotion products procured from online cosmetic shops may be attributed to the addition of potentially harmful dyes designed to enhance the visual appeal of the product and attract consumer interest through its vibrant appearance.

3.2. Qualitative test

Qualitative testing examines and identifies the compounds within the sample under analysis (Siyoto & Sodik, 2015). A 96% ethanol solution is utilized as the solvent due to its polar nature, which facilitates absorption by the stationary phase, which is silica gel (Primadiamanti *et al*., 2018). The sample solution was subsequently filtered through filter paper to minimize the presence of small particles. The resulting filtrate was then used as the test or sample solution for further analysis.

In this study, silica gel GF254 was utilized as the stationary phase due to its ability to produce distinct color development when exposed to UV light at a wavelength of 254 nm. It exhibits excellent fluorescence under such conditions, making it suitable for this analytical application (Primadiamanti *et al*., 2018). The mobile phase, or eluent, comprised an 8:2 mixture of glacial acetic acid and toluene. Glacial acetic acid, known for its strong polar properties, was combined with toluene, an aromatic hydrocarbon exhibiting lipophilic characteristics. This

solvent combination was chosen because it offers an adjustable elution power, facilitating optimal separation during the analytical process.

After the sample was applied to the TLC plate, it was placed into the chamber and eluted until the solvent ascended to a predetermined upper limit marked on the plate. Once the solvent front reached this line, the plate was removed from the chamber and allowed to dry. The spots were then examined under a 254 nm UV lamp. The spots were measured for the six samples and the hydroquinone reference standard, after which the Rf values of both the sample spots and the reference standard were calculated. The qualitative analysis's results focused on the Rf value, which was determined to be 0.75 for both the comparison standard and the six samples of body-whitening hand and body lotion. Based on this Rf value, it was confirmed that all six samples tested positive for the presence of hydroquinone.

In accordance with the regulation outlined by the Head of the Indonesian Food and Drug Authority (BPOM RI) No. HK.03.1.23.08.11.07331 in 2011, the acceptable safety threshold for the hydroquinone analysis method using TLC stipulates an Rf value within the range of 0.2-0.3 (BPOM RI, 2011). However, the Rf values of the six samples analyzed exceeded the prescribed safety threshold. Subsequently, the samples that underwent qualitative analysis were subjected to quantitative analysis using the UV-Vis spectrophotometry method, which had previously been validated for accuracy and reliability.

3.3. Method validations

Method validation is essential to demonstrate that specific parameters meet the necessary requirements for their intended application. This procedure entails assessing multiple parameters via a sequence of laboratory studies. Prior to evaluating various validation parameters, it is essential to optimize the test circumstances to ascertain the maximum wavelength and operational duration.

The objective of determining the maximum wavelength is to find the absorption region that produces the absorbance value of the measured standard solution or to ascertain the optimal absorbance of hydroquinone. The maximum wavelength was determined using UV-Vis spectrophotometry within the 200-400 nm range. A hydroquinone standard solution with a concentration of 14 ppm was utilized, with methanol as the blank. The peak absorbance occurred at a wavelength of 295 nm, resulting in an absorbance value of 0.444. This corresponds with the theoretical maximum wavelength for hydroquinone, expected to be between 287 and 295 nmThe determination of the maximum wavelength is intended to identify

the region of absorption that yields the absorbance value of the measured standard solution or to establish the optimum absorbance of hydroquinone (Adriani & Safira, 2019).

The operation time denotes the period required for all analytes to completely react with the reagents. This timing is crucial for determining the duration necessary for hydroquinone to reach a steady absorbance level. The operational duration was ascertained utilizing a standard solution with a concentration of 14 ppm, monitored at a wavelength of 295 nm across a time span of 0 to 30 minutes. The curve (**Figure 1**) demonstrates that the assessment of operation time reveals a stability of hydroquinone absorbance between 15 and 20 minutes, consistently recorded at 0.541. Accordingly, based on the findings from the operating time evaluation, this study designates the 15th minute as the chosen operating time.

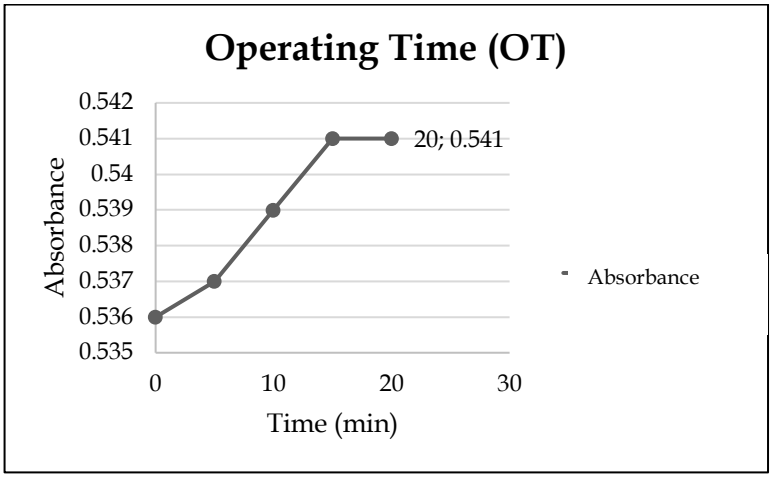

Figure 1. The curve of the correlation of time to the stability of the absorbance

The first parameter in method validation is linearity, which refers to the capacity of an analytical method to produce a response that is directly proportional to the concentration of the analyte within the sample, either inherently or through the application of an appropriate mathematical transformation (Irnawati, *et al*., 2016). Standard curves and linearity assessments were established using hydroquinone standard solutions at 10, 14, 18, 22, and 26 ppm concentrations. These solutions were prepared by diluting a 100 ppm hydroquinone stock solution to 50 ppm, with methanol as the blank. Subsequently, measurements were conducted at the maximum wavelength, and the linearity evaluation was replicated three times to ensure accuracy. According to the measurement data for the hydroquinone standard solution obtained in this study, as presented in **Figure 2**, it is evident that an increase in the concentration of the standard solution corresponds to a higher absorbance value (Nurfitriani, *et al*., 2015).

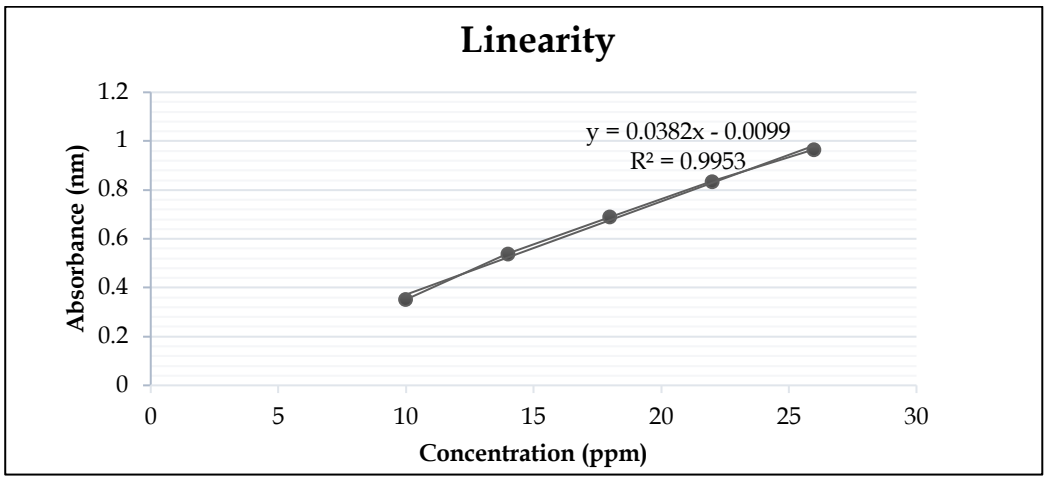

Figure 2. Concentration vs absorbance correlation curve

The next parameters in method validations were the limit of detection (LOD) and the limit of quantification (LOQ). The detection limit represents the minimum quantity of analyte within a sample that can be identified while still producing a measurable response. Conversely, the quantitation limit serves as an analytical parameter, indicating the smallest quantity of analyte that can be accurately quantified while satisfying criteria for precision and reliability. The LOD and LOQ are both based on the linear equation of the standard curve and are determined by statistics. The LOD and LOQ (**Table 1**) were established through a calibration curve derived from standard solutions at concentrations of 10, 14, 18, 22, and 26 ppm, each concentration replicated three times. A calibration curve was constructed from the mean values of these three replications.

Within this study, the LOD was determined to be 0.3862 g/mL (as shown in **Table 1**), indicating that, at this concentration, sample measurements remain feasible and yield results that reflect the instrument's accuracy in accordance with the specific accuracy level of the analytical outcomes (Tulandi, *et al*., 2015). Should the concentration of hydroquinone measured in the sample exceed 0.3862 g/mL, the resulting signal can be reliably attributed to hydroquinone itself. On the other hand, if the measured concentration is less than 0.3862 g/mL, it is not possible to be sure that the signal is from hydroquinone (Yulia & Ismi, 2020).

The LOQ value of 1.2872 g/mL (shown in **Table 1**) means that measurements at this concentration can still give accurate analytical results. Therefore, the results may be deemed accurate if the measured concentration is no less than 1.2872 g/mL (Yulia & Ismi, 2020).

Table 1. Result of LOD and LOQ

Data	Result
Σ	0.00021978
a	-0.009919
b	0.03815
S(y/x)	0.004917
LOD	0.3862
LOQ	1.2872

Next, precision and accuracy were also validated. Precision refers to the degree of consistency or repeatability of an analytical method. It reflects the method's accuracy when the same analysis is performed repeatedly under identical conditions within short intervals of time (Riyanto, 2014). The precision was assessed through repeatability tests using a standard solution at a concentration of 26 ppm, with precision determined over six replicates (**Table 2**). With the calibration curve represented by the equation *y = 0.0382x - 0.0099*, the standard deviation (SD) has been calculated as 0.2554, while the relative standard deviation (RSD) is determined to be 1.912% (as shown in **Table 2**).

Precision is indicated by the Relative Standard Deviation (RSD) value; a lower RSD signifies greater accuracy, whereas a higher RSD denotes reduced accuracy (Chakti *et al*., 2019). For precision to meet validation standards, an RSD value of 2% or less is required (Harmita, 2004). The RSD acquired in this study met the validation standards for the precision method.

The accuracy test aims to evaluate the closeness of the analytical results to the true analyte value present in the sample. A recovery test was performed to assess the extent of hydroquinone loss during the assay process. This study employed the addition method (standard addition) to determine the accuracy value, where the sample was first analyzed, followed by the addition of a known quantity of the analyte (pure analyte/standard) to the sample, which was then mixed and re-analyzed. The results of this accuracy assessment are presented in **Table 3**.

Within this study, recovery calculations were conducted to ensure that the concentrations measured corresponded closely to the actual concentrations present in the sample. An average recovery percentage of 96.77% was obtained (refer to **Table 3**), suggesting that the results are within an acceptable recovery range. The recovery percentage indicates the degree of accuracy, with an acceptable accuracy range defined as 80 to 110% (Harmita, 2004).

Table 2. Result of precision

R	Abs	x	$\bar{x}$	x - $\bar{x}$	$(x - \bar{x})^2$
1	0.431	11.5419		-1.815	3.2942
2	0.461	12.3272		-1.029	1.0603
3	0.484	12.9293		-0.4276	0.1828
4	0.507	13.5314	13.3569	0.1745	0.0305
5	0.542	14.4476		1.0907	1.1896
6	0.577	15.3638		2.0069	4.0276
		Mean			1.6308
		SD			0.2554
		RSD (%)			1.912

Table 3. Result of accuracy

Standard Addition (ppm)	R	Measured concentration			% *Recovery*	Mean (%)
		Cf	Cu	Ca		
10	1	14.6309	6.3848	8.6099	95.77	94.06
	2	14.4738		8.5838	94.24	
	3	14.3691		8.6623	92.17	
18	1	22.0654		16.5942	94.49	95.18
	2	22.2749		16.6466	95.77	
	3	22.2225		16.6204	95.29	
26	1	27.8769		22.8246	94.16	93.81
	2	28.0340		22.8508	94.85	
	3	27.5485		22.9031	92.41	
			Σ			**94.35%**

3.4. Determination of hydroquinone levels in samples

Samples exhibiting positive outcomes in the qualitative test subsequently confirmed hydroquinone's presence upon quantitative analysis, thereby reinforcing the initial findings. The determination of hydroquinone levels within the sample commenced with the sample preparation process, employing methanol as the solvent. Each sample was weighed to 100 mg, dissolved in 50 mL of methanol, and filtered using Whatman paper. This filtration step during preparation was essential for isolating hydroquinone from other compounds present in the lotion, including the lotion base and any additional active ingredients contained within it. The concentration of hydroquinone in the sample was determined at a maximum wavelength of 295 nm with an operating time set at 15 minutes. Each sample measurement was replicated three times to enhance accuracy. The absorbance measurement results for all six samples are presented in **Table 4**.

Table 4. Result of hydroquinone content

Sample	Abs (mean)	C (ppm)	%C
1	0.022	0.8350	0.0418
2	0.0243	0.8953	0.0448
3	0.0177	0.7225	0.0361
4	0.0117	0.5654	0.0283
5	0.018	0.7304	0.0365
6	0.234	6.3848	0.3193

According to the measurements and calculations presented in **Table 4**, all six samples were found to contain hydroquinone. Samples 1, 2, 3, 4, and 5 had hydroquinone level of 0.8350, 0.8953, 0.7225, 0.5654, and 0.7304 ppm, respectively. The average concentration across these samples exceeded the LoD of 0.3862 ppm but remained below the LOQ of 1.2872 ppm. Thus, while hydroquinone is positively identified in these body whitening hand and body lotion samples, the concentration is too low for precise quantification. Conversely, sample 6 contained hydroquinone at a concentration of 6.3848 ppm, exceeding the LOD and LOQ, confirming hydroquinone's presence with sufficient measurement accuracy.

The quantitative test results reveal that certain whitening hand and body lotions still contain hydroquinone despite its prohibition under BPOM Regulation Number 18 of 2015, which outlines the technical requirements for cosmetic ingredients. According to this regulation, hydroquinone is banned in cosmetic products, except for artificial nail preparations, where it is permitted at a maximum concentration of 0.02% (BPOM RI, 2015).

Based on the findings of this research, the following recommendations are proposed: (1) For the public, particularly women of all ages, it is advised to exercise caution when selecting whitening hand and body lotions available through online retailers. Consumers should opt for products that display their composition, registration number, manufacturer's details, batch number, and BPOM registration status; (2) It is recommended that government authorities engage in public outreach to educate communities on the potential risks associated with whitening hand and body lotions containing hydroquinone, thereby encouraging more informed purchasing choices; (3) Future researchers are encouraged to investigate hydroquinone content in other brands of whitening hand and body lotions, including those available in offline retail outlets.

4. Conclusions

Based on the findings from the research conducted on six samples of whitening hand and body lotions available in online shops, the following conclusions can be drawn:

1. The qualitative analysis of hydroquinone, conducted using Thin-Layer Chromatography (TLC), indicated that all six samples tested positive for the presence of hydroquinone. This identification was based on the samples' Rf values, which matched the reference standard Rf value of 0.75.
2. The UV-Vis spectrophotometry method demonstrated strong linearity, with a determination coefficient of 0.9953 and a correlation coefficient of 0.99764. Precision testing yielded an RSD value of 1.912%, while accuracy, reflected in the average recovery percentage, was 94.35%. Additionally, the method exhibited a Limit of Detection (LOD) of 0.3861 µg/mL and a Limit of Quantitation (LOQ) of 1.2872 µg/mL. These method validation results indicate that the approach employed for testing hydroquinone in body whitening hand and body lotions is suitable, as it meets the required validation criteria.
3. The quantitative analysis of hydroquinone levels across six samples of whitening hand and body lotion revealed concentrations ranging from 0.0283 to 0.3193 µg/mL. Among these, sample 4 contained the lowest concentration of hydroquinone, while the highest concentration was detected in sample 6.

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