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ANALYSIS OF HYDROQUINONE IN FACE WHITENING CREAM CIRCULATING IN SEMARANG CITY USING UV-VISIBLE SPECTROPHOTOMETRY METHOD

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ABSTRACT

Background: The Indonesian Food and Drug Authority (BPOM) issued a regulation stating that any hydroquinone preparation with a concentration of more than 2% is categorized as a prescription drug (List G Drug). Therefore, it should only be given with a doctor's prescription.

Aim: This study aims to assess the validity of the UV-visible spectrophotometric method for hydroquinone analysis, measure the hydroquinone content in whitening creams sold in Semarang, and determine whether they comply with BPOM regulations.

Method: A total of 10 samples of face whitening creams obtained from cosmetic shops located in North, East, West, Central, and South Semarang were subjected to this study. The validation parameters to be tested were linearity, Limit of Detection (LoD), Limit of Quantitation (LoQ), precision, and accuracy.

Result: The linear regression equation obtained was y=0.0132x+0.0655 with r=0.9986 and R^2=0.9973. This method showed high precision with a %RSD of 0.84%, LoD and LoQ values of 2.5 µg/mL and 8.3 µg/mL, and an accuracy value of 100.211%. The hydroquinone content in samples A, G, H, and J could not be determined because they were not detected. Hydroquinone content in creams B, C, D, E, F, and I were 0.84%, 4.77%, 0.74%, 2.27%, 4.75%, and 1.39%, respectively.

Conclusion: The results showed that the UV-visible spectrophotometric method was valid for hydroquinone analysis in samples of face whitening cream for all tested parameters.

Keywords: Hydroquinone, UV-vis Spectrophotometry, Validation of Analytical Method, Face Whitening Cream

INTRODUCTION

The Food and Drug Administration (FDA) regulates that all hydroquinone-containing topical products should only be given with a doctor's prescription (Food and Drug Administration, 2006). On the other hand, the Indonesian Food and Drug Authority or *Badan Pengawas Obat dan Makanan* (BPOM) regulates that hydroquinone with concentrations of more than two percent is classified as a prescription drug (List G Drug) (Badan POM RI, 2007). These two regulations were issued because the use of hydroquinone without supervision may cause side effects such as irritation, skin redness, and a burning sensation. Besides, it may cause severe adverse effects as well, such as abnormalities in the kidneys (nephropathy), blood cancer (leukemia), and liver cell cancer (hepatocellular adenoma).

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However, in practice, many manufacturers formulate hydroquinone as an over-the-counter cream with a content of more than 2%. For example, Astuti *et al.* (2016) conducted research on assaying hydroquinone in face whitening creams sold in minimarkets in Yogyakarta. It showed that 64.29% of whitening creams contained hydroquinone, with 88.89% of them containing hydroquinone levels above 2%. Apart from that, research was also conducted by Harimurti *et al.* (2021) regarding the identification of the hydroquinone content in whitening creams sold in traditional markets in Banjarnegara. The results showed that 6 out of 21 samples of whitening creams contain hydroquinone. In 6 samples containing hydroquinone, the lowest level was 0.06%, and the highest was 11.18%. Based on those findings, it is necessary to research the analysis of hydroquinone in whitening creams marketed in other regions, such as Semarang. This study aims to determine the validity of the UV-Vis spectrophotometric method for hydroquinone analysis, to assay the hydroquinone in whitening creams sold in Semarang City, and to ascertain whether these levels comply with Indonesian Food and Drug Authority (BPOM) regulations.

MATERIAL AND METHODS

This research was conducted at the Research Laboratory of the Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang. A hydroquinone standard solution of 50 µg/mL was prepared by dissolving 5 mg of pure hydroquinone in 100 mL of methanol. Standard curves were made by diluting hydroquinone standard solution into concentrations of 1, 14, 20, 28, 32 and 48 µg/mL. The maximum wavelength was determined from hydroquinone 15 µg/mL in the wavelength range of 200 – 400 nm. A standard curve was produced by plotting concentration vs absorbance (Ernawati *et al.*, 2016). To validate the analysis method, accuracy, precision, linearity, Limit of Detection (LoD), and Limit of Quantitation (LoQ) were tested.

Accuracy Test

The sample solution was prepared by weighing 10 mg of face whitening cream sample, and then standard hydroquinone was added according to Table 1. Then, methanol was added to 20 mL and shaken. Then the test solution was measured its absorption at the maximum wavelength using UV-vis spectrophotometry. This test was replicated 3 times.

Table 1. The Preparation of Accuracy Test

Solution concentration	Sample weight (mg)	Volume solution standard 50 µg/mL (mL)	Methanol volumes (mL)
14 µg/mL	10	5.6	Ad 20
22 µg/mL	10	8.8	Ad 20
30 µg/mL	10	12	Ad 20

The recovery value was calculated from the results of concentration levels of 14 µg/mL, 22 µg/mL, and 30 µg/mL. This value was used as the accuracy value. Percent recovery can be calculated using the formula in the following equation (Harmita, 2004):

$$R = \frac{Cf - Ca}{Ca *} x\ 100\%$$

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R = *percent recovery*
Cf = *measured total concentration*
Ca = *true sample concentration*
*Ca** = *added analyte concentration*

Precision Test

Each sample of whitening cream was weighed 10 mg and dissolved in 20 mL of methanol. The solution was filtered using filter paper. As much as 100 µL of filtrate was measured its absorbance using UV-vis spectrophotometry. Each sample was replicated 6 times. Standard deviation (SD) and Relative Standard Deviation (RSD) values were calculated using the following equation (Helwandi, 2016):

$$SD = \sqrt{\frac{\sum(xi - \bar{x})^2}{n-1}}$$

xi = *single measurement*
$\bar{x}$ = *average*
n = *number of measurements*

$$RSD = \frac{SD}{\bar{x}} \times 100\%$$

SD = *standard deviation*
$\bar{x}$ = *average*
RSD = *relative standard deviation*

Linearity Test

Solution with concentrations of 1, 14, 20, 28, 32, and 48 µg/mL were prepared from a standard solution of 50 µg/mL. Each solution was measured for its absorbance. Then, the relationship between concentration and absorption value was carried out in order to obtain a linear regression equation y = ax + b (Irnawati & Dewi, 2016).

Limit of Detection (LoD) and Limit of Quantitation (LoQ) Determination

From the linear equation of the calibration curve, a single absorbance (xi) and average absorbance ($\underline{xi}$) were obtained. LoD and LoQ values were calculated with the following formula (Wardani, 2012)

LoD = 3 x (SD/S)
LoQ = 10 x (SD/S)

SD = standard deviation
S = slope

Determination of Hydroquinone Levels in Whitening Cream

As much as 10 mg of whitening cream from each sample was dissolved in 20 mL of methanol. It was taken 100 µl and read for its absorbance using a UV-Vis spectrophotometer at the maximum wavelength. The concentration of hydroquinone was calculated using the regression equation obtained previously (Irnawati & Dewi, 2016).

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RESULTS AND DISCUSSION

The maximum wavelength of hydroquinone was 294 nm with an absorbance value of 0.264 (Figure 1, Table 2). Determination of the maximum wavelength in this study was carried out at intervals of 289-300 nm, referring to research conducted by Irnawati *et al.* (2016), which stated that hydroquinone in methanol has a maximum wavelength of 293 nm.

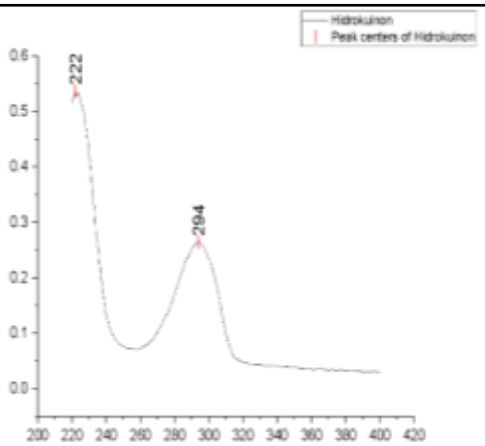

Figure 1. The spectrum of hydroquinone in wavelength range of 200 – 400 nm

Table 2. Maximum wavelength determination

Wavelength	Absorbance
289 nm	0.245
290 nm	0.252
291 nm	0.257
292 nm	0.261
293 nm	0.263
294 nm	0.264
295 nm	0.263
296 nm	0.26
297 nm	0.255
298 nm	0.249
299 nm	0.242
300 nm	0.234

The hydroquinone standard curve was prepared in this study by preparing several hydroquinone concentrations, including 1 µg/mL, 14 µg/mL, 20 µg/mL, 28 µg/mL, 32 µg/mL, and 48 µg/mL. These concentrations were chosen so that the absorbance value ranged from 0.2 to 0.8. The hydroquinone standard curve in this study showed a linear relationship between absorbance and concentration, indicated by the r^2 value of 0.9973 with the regression equation y = 0.0132x + 0.0655. This regression equation is used to determine the hydroquinone content in the whitening cream sample.

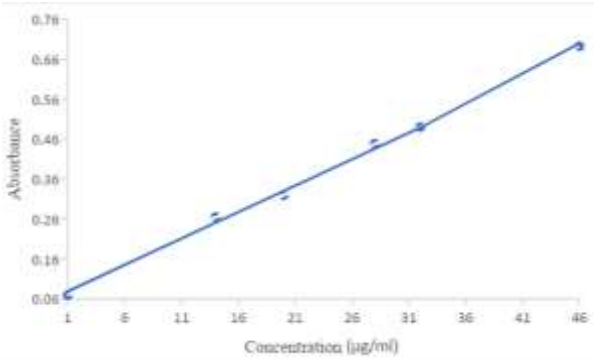

Figure 2. Hydroquinone standard curve

Table 3. The absorbance of each concentration of hydroquinone standard solution

Conc. (µg/mL)	Absorbance			Average
	1	2	3	
1	0.068	0.069	0.07	0.069
14	0.264	0.264	0.266	0.2647
20	0.319	0.32	0.319	0.3193
28	0.481	0.432	0.43	0.4477
32	0.5	0.483	0.486	0.4897
48	0.693	0.693	0.69	0.692

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Accuracy

Accuracy is defined as how close the results of tests are to the true value. When a standard amount of analyte is fixed to a sample, accuracy is determined as the percentage of recovery (Susilo *et al.*, 2022). The average percentage of recovery of whitening cream samples was 100.21% (Table 4). The accuracy of this method was acceptable because it falls within the required range of 97 – 103% (Wardani, 2012).

Table 4. The Percentage of Recovery

Sample	Measured Concentration			Theoretical Concentration	% Recovery		
	14 µg/mL	22 µg/mL	30 µg/mL		14 µg/mL	22 µg/mL	30 µg/mL
B	18.144	25.947	3.760	4.2	99.597	98.85	101.2
C	37.942	45.821	53.927	23.977	99.749	99.29	100.674
D	17.487	25.644	33.952	3.75	99.026	99.518	100.674
E	25.568	33.876	42.159	11.427	101.008	102.042	102.44
F	37.727	46.275	53.371	23.902	101.209	101.697	98.231
I	21.073	28.725	37.664	7.083	99.903	98.372	101.937
Average					100.082	99.962	100859

Precision Test

Precision is the degree of consistency of individual test results when the procedure is repeated on multiple samples or homogeneous samples. In this study, the precision test was conducted using repeatability analysis in which the analysis was carried out by the same analyst on the same day in the same laboratory (Eserian & Lombardo, 2015). The results showed that the % RSD value obtained from 6 replications was below 2%. The highest % RSD was shown by sample H, with a value of 1.63, and the lowest %RSD was shown by sample C, with a value of 0.49 (Table 5). Samples A, B, C, E, F, G, I, and J have a very precise level of accuracy because the RSD value is below 1%. Sample D and sample H have a high level of accuracy because the RSD values are above 1% and below 2%. The average value of the percentage of RSD in the analytical method in this study was 0.84%. Hence, this analytical method is precise.

Table 5. The Percentage of Relative Standard Deviation

Sample	% RSD	Average of % RSD
A	0.87	
B	0.96	
C	0.49	
D	1.19	
E	0.91	
F	0.79	0.84%
G	0.69	
H	1.63	
I	0.76	
J	0.96	

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Linearity Test

Linearity is the ability to give test results that are strictly proportional to the concentration of the analyte in the sample within a certain range (Fahira *et al.*, 2021). The linearity of the hydroquinone standard curve was determined from a concentration range of 1 to 48 µg/mL using pro-analytical methanol and read at a wavelength of 294 nm. The relationship between concentration and absorbance shows a linear regression equation, namely y = 0.0132x + 0.0655, with a value of r^2 = 0.9973 (Figure 2). From this equation, it can be seen that the correlation coefficient is 0.9986. A method has good linearity if the correlation coefficient value is more than 0.995 and the coefficient of determination is more than 0.990 (Lesnussa & Dulanlebit, 2019)

Limit of Detection (LoD) and Limit of Quantitation (LoQ) Determination

The Limit of Detection (LoD) is the smallest concentration of analyte that can be detected in a sample. The limit of Quantitation (LoQ) is the lowest concentration of an analyte in a sample that can be determined and meets the requirements of precision and accuracy. Limits of detection and quantitation are calculated statistically through a linear equation from the standard curve (Shrivastava & Gupta, 2011). The calculation resulted in a LoD value of 2.5 µg/mL and a LoQ value of 8.3 µg/mL (Table 6).

Table 6. LoD and LoQ Values

Concentration (µg/mL)	**Absorbance average (y)**	**y'**	**y-y'**	**$(y-y')^2$**
1	0.069	0.080	-0.011	0.000121
14	0.265	0.251	0.014	0.000196
20	0.319	0.330	-0.011	0.000121
28	0.448	0.436	0.012	0.000144
32	0.490	0.489	0.001	0.000001
48	0.692	0.700	-0.008	0.000064
$\sum(y-y')^2$				0.000647
$\sum(y-y')^2/n-1$				0.0001294
SD				0.011
LoD				2.5 µg/mL
LoQ				8.3 µg/mL

The UV-Vis spectrophotometric method was valid for analyzing hydroquinone in face whitening cream for parameters of linearity, the limit of detection, the limit of quantitation, precision, and accuracy. The summary of the analysis is presented in Table 7.

Table 7. Summary of Method Validation Results

Parameters	**Results**	**Requirement**
Linear regression equation	y= 0.0132x + 0.0655	-
Linearity	r= 0.9986, R^2=0.9973	R^2 is close to number 1
LoD and LoQ	2.5 µg/mL; 8.3 µg/mL	-
Precision	0.84%	<2%
Accuracy	100.211%	97 – 103%

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Determination of Hydroquinone Levels in Face Whitening Cream Samples

Determination of the hydroquinone content in the sample was conducted qualitatively and quantitatively. The qualitative test aims to identify the presence or absence of hydroquinone content in the sample. It was carried out by looking at and comparing the spectrum produced by each sample to the standard spectrum of hydroquinone. Samples A, G, H, and J were identified as not containing hydroquinone. It means these samples did not contain hydroquinone at all, or the samples contained hydroquinone with a concentration below the LoD value, which was 2.5 µg/mL. Samples with codes B, C, D, E, F, and I were identified as containing hydroquinone (Figure 3).

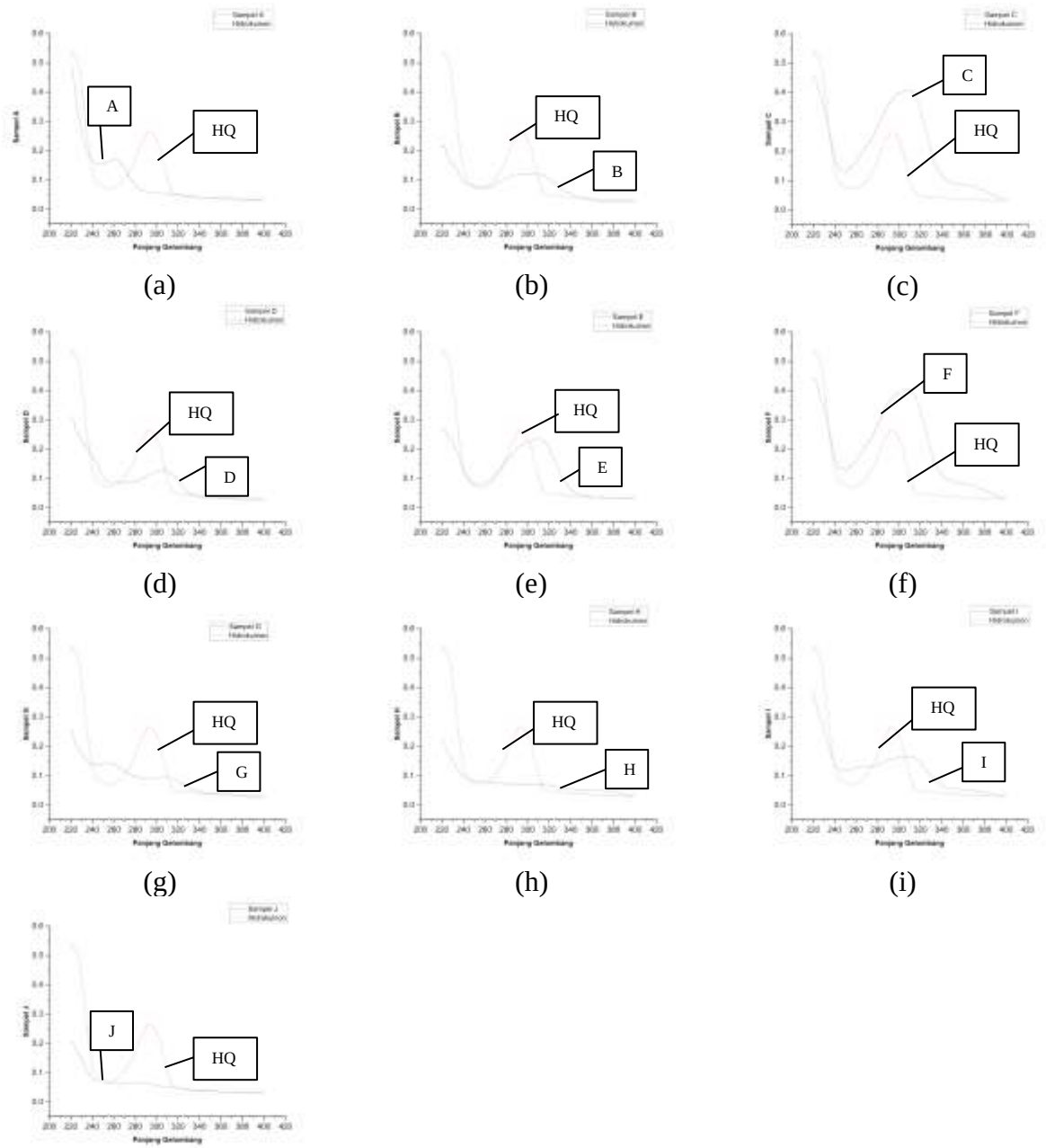

Figure 3. The spectrum of a sample of face whitening cream and standard hydroquinone (HQ) in sample A-J

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The results of determining the levels of hydroquinone in the face whitening cream samples showed that samples A, G, H, and J had levels below the detection and quantification limits of the spectrophotometer used, namely 2.5 µg/mL and 8.3 µg/mL, so that the level cannot be determined because it cannot be detected by the spectrophotometer used (Table 8).

Table 8. Results of Determination of Hydroquinone Levels in Samples of Face Whitening Cream

Sample	Meet the requirement of BPOM (√ / –)	Level (µg/mL)	Level (%)
A	√	not detected	not detected
B	√	4.1919	0.84
C	–	23.8636	4.77
D	√	3.69949	0.74
E	–	11.3636	2.27
F	–	23.76263	4.75
G	√	not detected	not detected
H	√	not detected	not detected
I	–	6.9823	1.39
J	√	not detected	not detected

Samples B, D, and I showed concentrations above LoD but below LoQ; hence, the accuracy of the results could not be guaranteed. Hydroquinone content in samples B, D, and I was 4.192 µg/mL, 3.699 µg/mL, and 6.982 µg/mL. The percentage of hydroquinone content in samples of face whitening creams B, D, and I was 0.84%, 0.74%, and 1.39%, respectively (Table 8). These samples met Indonesian Food and Drug Authority (BPOM) requirements as over-the-counter creams with hydroquinone levels below 2%.

The hydroquinone content in samples C, E, and F were 23.864 µg/mL, 11.364 µg/mL, and 23.763 µg/mL, respectively. The percentage of hydroquinone content in samples C, E, and F each had a value of 4.77%, 2.27%, and 4.75% (Table 8). The percentage levels of these three samples did not meet Indonesian Food and Drug Authority (BPOM) requirements as over-the-counter face whitening creams because they contain hydroquinone with levels above 2%. These samples are categorized as prescription drugs that must be given with a doctor's prescription (BPOM RI, 2007).

Samples A, B, D, G, H, and J are registered by BPOM. These samples met the requirements of over-the-counter creams because the hydroquinone content in the cream was below 2%. Meanwhile, the samples C, E, F, and I are not registered by BPOM. Samples C, E, and F contained hydroquinone above limits; hence, they should not have been allowed to be sold freely, namely samples C, E, and F. Sample I was a sample that was not registered with BPOM. Still, the hydroquinone content was below 2% and met BPOM requirements as an over-the-counter cream.

CONCLUSION

Based on the research results, it can be concluded that the hydroquinone analysis method in face whitening cream meets the validation parameters, namely linearity, LoD, LoQ, precision, and accuracy. The obtained linear regression equation was y=0.0132x+0.0655 with r= 0.9986 and R^2= 0.9973, the precision was 0.84%, the LOD and LOQ values were 2.5 µg/mL and 8.3 µg/mL, and the accuracy value was 100.211%. The results of the qualitative test showed that 6 of the 10 samples analyzed contained hydroquinone. The results of the quantitative test showed that the percentage levels of hydroquinone in samples B, C, D, E, F, and I were 0.84%, 4.77%, 0.74%, 2.27%, 4.75%, 1.39%. Based on the results of determining the hydroquinone content in the whitening cream samples, it can be seen that of the 6 samples containing hydroquinone; there were 3 samples with hydroquinone levels above 2%, so they did not meet the requirements as over-the-counter creams according to BPOM regulations.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

AUTHOR DETAILS

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