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Analysis of Hydroquinone and Mercury in Whitening Cream Using UV-Vis Spectrophotometry and ICP-OES

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ABSTRAK

Krim pemutih mengandung zat aktif yang menghambat pembentukan melanin. Hidrokuinon dan merkuri sering ditambahkan karena efektivitasnya dalam mengurangi melanin dan memberikan efek kulit halus secara cepat. Penelitian ini bertujuan untuk mengetahui kadar hidrokuinon dan merkuri dalam krim pemutih menggunakan spektrofotometri UV-Vis. Pengambilan sampel secara acak dari toko kosmetik di Kuningan menghasilkan 9 sampel krim pemutih. Metode eksperimen mencakup pengumpulan sampel, pembuatan larutan standar, pembuatan kurva kalibrasi, serta uji kualitatif untuk hidrokuinon dan merkuri; $FeCl_3$ digunakan untuk uji hidrokuinon, dan uji warna kalium iodida (KI) untuk merkuri. Analisis kuantitatif menggunakan ICP-OES dan Spektrofotometri UV-Vis. Hasil uji kualitatif dengan KI menunjukkan bahwa 6 dari 9 sampel (A, B, C, D, F, I) mengandung merkuri. Uji $FeCl_3$ menunjukkan adanya hidrokuinon pada 3 sampel (D, E, G). Spektrofotometri UV-Vis mendeteksi hidrokuinon pada 8 sampel (A, B, C, D, E, F, H, I) dengan kadar melebihi 0,02%, berkisar antara 0,0809% hingga 3,5875%. Sampel G mengandung 0,0134%. Uji ICP-OES menunjukkan kandungan merkuri pada sampel A–H berkisar antara -0,0071 hingga -0,6018 mg/L, atau kurang dari 0,0001% dalam mg/Kg, namun sampel I menunjukkan kandungan merkuri tinggi sebesar 138,4844 mg/L.

Kata kunci: hidrokuinon · ICP-OES · krim pemutih · merkuri · spektrofotometri UV-Vis

ABSTRACT

Whitening creams contain active substances that inhibit melanin formation. Hydroquinone and mercury are often added due to their effectiveness in reducing melanin and providing quick skin smoothing. This study aims to determine the levels of hydroquinone and mercury in whitening creams using UV-Vis spectrophotometry. A random sampling from cosmetic stores in Kuningan yielded 9 whitening cream samples. The experimental method included sample collection, standard solutions preparation, calibration curve construction, and qualitative tests for hydroquinone and mercury. $FeCl_3$ was used for hydroquinone, and potassium iodide (KI) color test for mercury, with ICP-OES and UV-Vis Spectrophotometry for quantitative analysis. Qualitative KI testing showed that 6 out of 9 samples (A, B, C, D, F, I) contained mercury. $FeCl_3$ testing found hydroquinone in 3 samples (D, E, G). UV-Vis Spectrophotometry identified hydroquinone in 8 samples (A, B, C, D, E, F, H, I) exceeding 0.02%, with concentrations ranging from 0.0809% to 3.5875%. Sample G had 0.0134%. ICP-OES testing revealed mercury content from -0.0071 to -0.6018 mg/L in samples A-H, less than 0.0001% in mg/Kg, but sample I had a high mercury content of 138.4844 mg/L.

Keywords : *hydroquinone · ICP-OES · mercury · whitening cream · UV-VIS spectrophotometry*

INTRODUCTION

Whitening cream is a cosmetic preparation containing active substances that can inhibit the formation of melanin pigment on the skin (Ramadhan, et al., 2024). Two commonly added chemicals in cosmetics are hydroquinone and mercury, due to their ability to inhibit melanin formation on the skin's surface and make the skin smoother in a relatively short time (Neytal, et al., 2024). Mercury is one of the hazardous substances found in facial and skin whitening creams. Mercury is a liquid silver metal or commonly known as mercury (Sable, et al., 2024). Heavy metals, when accumulated in an organism's body, can inhibit enzyme function, disrupting the metabolic process, and can even trigger and cause allergies, mutagenesis, teratogenicity, or carcinogenicity in humans (Saravanan, et al., 2024). Cream-based cosmetics containing hydroquinone are widely used to remove dark spots on the

face (Hamie, et al., 2024). The whitening effect of hydroquinone on the face is very slow and will be faster with a higher concentration, but a very high concentration will have undesired effects (Harnchoowong, et al., 2024). Hydroquinone is a chemical substance used to eliminate hyperpigmentation on the skin with the goal of skin whitening and inhibiting melanin production on the skin (Ferrarini, et al., 2024). Prolonged use of hydroquinone can also lead to cancer symptoms, kidney abnormalities, cell proliferation, and the potential for carcinogenic and teratogenic effects (Wang, et al., 2024). The method used in this study is an experimental method with research stages, including sample collection, preparation of hydroquinone and mercury standard solutions, calibration curve preparation, qualitative testing of hydroquinone with $FeCl_3$, qualitative testing of mercury with the KI color test. Testing the mercury content using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) and UV-Vis Spectrophotometry Shimazu 1900. Regulation Number: HK.00.05.42.1018 of 2008 on cosmetic ingredients and Regulation Number: HK.03.1.23.08.11.07517 of 2011 on technical requirements for cosmetic ingredients prohibit the use of mercury in cosmetics (BPOM, 2011).

METHODS

Materials and instrumentation

The equipment used in this research consists of an analytical balance, spatula (*Pyrex*), stirring rod, volumetric pipet (*Pyrex*), 100 mL, 50 mL, 10 mL measuring flasks (*Newtech*), filter paper (*Whatman*), funnel (*Herma*), cuvette, micropipette, glass beaker (*Pyrex*), dropping plate, and UV-visible spectrophotometer (*Shimazu*), porcelain crucible, analytical balance (Henher), Bunsen burner, test tube, Erlenmeyer flask (*Pyrex*), measuring flask, Kjeldahl flask, electric heater, stand, ICP-OES spectrometry.

The materials used in this research include random samples of whitening cream taken from Kuningan District, $FeCl_3$ reagent, 4N HCl (Merck), 96% ethanol (Merck), hydroquinone standard solution, methanol (Merck), deionized water, 5M pro-analysis nitric acid, 1000 ppm mercury standard solution, and deionized water.

Research Procedure

1. Sampling

The sampling of whitening cream was conducted in the Kuningan city area. The sampling was based on the random sampling technique. Random sampling is a method in which samples are taken from a population without considering strata within the population, and each member of the population has an equal chance of being selected as a sample (Miller & Miller, 2010). The samples were randomly obtained from cosmetic stores in the Kuningan market, totaling 9 pieces.

2. Sample Preparation

Reflux Destruction

One gram of the whitening cream sample was weighed in a reflux flask, and then 5 mL of HNO_3: HCl (3:1) was added. It was heated by reflux for 3 hours. The sample was then cooled to room temperature. The sample solution was filtered using filter paper. The sample was dissolved in a 25 mL volumetric flask and made up to volume with Milli-Q, then homogenized. Subsequently, the sample is ready for measurement.

Preparation of Hg standard solution

The standard solution was diluted by pipetting 1 mL of the 1000 ppm Hg standard solution into a 10 mL volumetric flask. It was then made up to volume with Milli-Q, homogenized, and resulted in a 100 ppm standard solution. Various concentrations of the standard solution were prepared by pipetting 0.25, 0.5, 0.75, 1, and 1.25 mL of the 100 ppm standard solution into 10 mL volumetric flasks. These were made up to volume, marked, and homogenized to obtain concentrations of 1, 2, 3, 4, and 5 ppm (Shevla, 1985). The standard solutions were measured using Inductive Couple Plasma-Optical Emission Spectroscopy (ICP-OES) with the maximum wavelength to determine their absorbance values. The maximum wavelength for Hg was found to be 253-652 nm.

RESULT AND DISCUSSION

Qualitative Mercury Test Using KI Reagent

Qualitative testing for mercury with potassium iodide (KI) reagent involved the addition of concentrated HNO_3, aiming to dissolve the mercury metal due to mercury's propensity to react with concentrated HNO_3. The reaction of mercury metal in concentrated nitric acid solution is as follows:

$$Hg + 2NO_3^- + 4H^+ \rightarrow Hg^{2+} + 2NO_2 + 2H_2O$$

Samples containing mercury, specifically samples with codes A, B, C, D, F, and G, showed a positive result with the addition of KI solution, indicating a color change from red to black, which is HgI_2 (Mercury (II) Iodide). The reaction is as follows:

$$Hg^{2+} + 2KI \rightarrow 2HgI_2 + 2KI$$

The positive test results of the samples can be observed in Table 1.

Table 1. The results of the qualitative mercury test using KI reagent.

No	Sampel Code	Color Observation	Result
1	A	Orange with black precipitate	+
2	B	Brown with black precipitate	+
3	C	Brown with black precipitate	+
4	D	Brick red with black precipitate	+
5	E	Brown without precipitate	-
6	F	Brick red with black precipitate	+
7	G	Yellow	-
8	H	Orange	-

9	I	Orange with black precipitate	+

Qualitative Testing for Hydroquinone

The results obtained from the qualitative test for hydroquinone with 1% $FeCl_3$ reagent indicate that samples with codes A, D, and F tested positive for containing hydroquinone, exhibiting a dark brown color. Hydroquinone, when added to $FeCl_3$, forms a complex compound as the oxygen in hydroquinone bonds with $FeCl_3$, resulting in a reaction that produces a green to black color in an acidic environment. The results of the qualitative test for hydroquinone with $FeCl_3$ reagent are presented in Table 2.

Table 2. The results of the qualitative hydroquinone test using $FeCl_3$ reagent

No	Sample Code	Color Observation	Result
1	A	Reddish brown	-
2	B	Reddish brown	-
3	C	Reddish brown	-
4	D	Blackish Brown	+
s5	E	Blackish Brown	+
6	F	Reddish brown	-
7	G	Blackish Brown	+
8	H	Light Yellow	-
9	I	Reddish brown	-

Determination of Maximum Wavelength

The determination of the wavelength was conducted in the range of 256-293 nm, where Hydroquinone in a methanol solution obtained a maximum wavelength of 293 nm. The maximum wavelength of hydroquinone was obtained from measuring the absorbance of the standard solution at the wavelength of 293 nm. At a wavelength of 293 nm, 2 groups were detected. The first is that there are 3 chromophore groups, namely C=C. The second is the 2 ausochrome group, namely the 2 OH group. The ausochrome group is a group characterized by the presence of a lone pair of electrons attached to the chromophore group. And the chromophore group is a functional group that absorbs or absorbs electromagnetic radiation in the ultraviolet wavelength region and the visible light region. Chromophores are conjugated or alternating double bond structures. Examples of chromophores: C=O, C=C, N=N and NO_2. The results of the maximum absorbance measurements can be seen in Fig. 1.

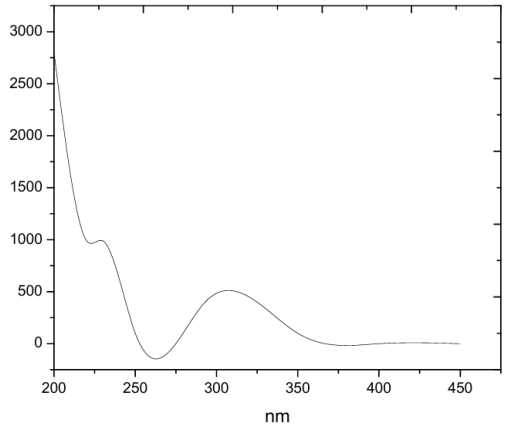

Figure 1. Maximun Wavelength of Hydroquinone in a methanol solution

Determination of the calibration curve for standard hydroquinone

Based on the determination of the calibration curve for standard hydroquinone with dilution concentrations of 2, 4, 6, 8, and 10 ppm, consecutive absorbance values were obtained as follows: 0.049; 0.124; 0.196; 0.244; and 0.303. Subsequently, a calibration curve of concentration against absorbance was generated, as shown in Fig. 2.

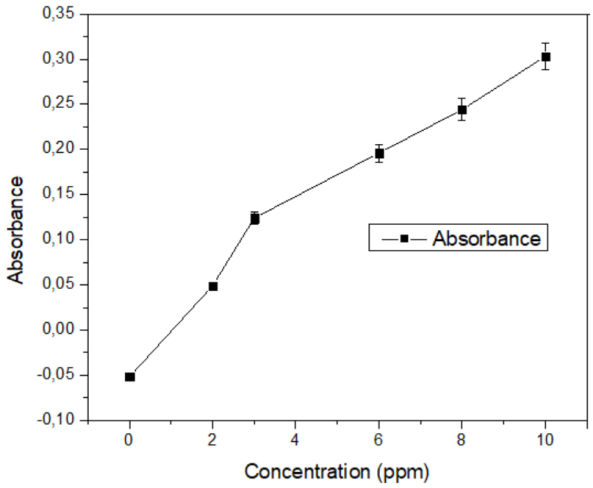

Figure 2. Standard hydroquinone calibration curve

Based on fig. 2, the curve forms a linear line, and the equation of the line is determined as y = 0.0345x – 0.0297, with a slope value of 0.0345, an intercept value of 0.985, and a regression value of 0.985. If the regression coefficient approaches the value of 1, it indicates a linear relationship between the concentration and the resulting absorbance. In other words, an increase in the analyte's absorbance values is directly proportional to the increase in its concentration, in accordance with the criteria for a good correlation coefficient (r) of 0.989. The absorbed light will be transferred to the atoms or molecules in the sample so that an electronic transition occurs. The amount of light absorbed/absorbance is proportional to the concentration of the analyte in the sample in accordance with the Lambert-Beer Law with the formula:

$$A = \varepsilon . t . C \quad (1)$$

Where:

A = Absorbance

ε = absorptivity molar (L/mol.cm)

t = thickness of the cuvette solution (cm)

C = solution concentration (ppm)

Hydroquinone Content Determination

The concentration results for samples testing positive for hydroquinone were obtained using UV-Vis Spectrophotometry. Samples indicating the presence of hydroquinone can be observed in Table 3.

Table 3. Hydroquinone Concentration in the Sample

No	Sample Code	Absorbance	Concentration (%)
1	A	0.557	0.1643
2	B	0.513	0.1524
3	C	0.500	0.1492
4	D	0.439	0.1023
5	E	0.528	3.5875
6	F	0.441	0.3140
7	G	2.157	0.0314
8	H	0.227	0.0809
9	I	0.408	0.1262

According to the regulations of BPOM (Indonesian National Agency of Drug and Food Control) based on the warning No. KH.03.1.23.08.11.07517 in 2011 regarding the technical requirements for cosmetic ingredients, Hydroquinone is prohibited for use as a whitening agent in cosmetics. Hydroquinone is only permitted for use in cosmetics for artificial nails with a concentration of 0.02% (Valenzuela, et al., 2024). Therefore, samples containing hydroquinone with codes A, B, C, D, E, F, H, and I exceed the threshold of 0.02%. Sample with code G is still within the permissible limit, below 0.02%.

Mercury Content Determination

The concentration results for testing positive mercury were obtained using ICP-OES (Inductively Coupled Plasma - Optical Emission Spectroscopy). Prior to measuring the mercury concentration in the samples, the maximum wavelength for mercury was first determined. The obtained line equation is y = 711.5x + 15.3, and mercury is located at the maximum wavelength of 253.652 nm. The determination of the maximum wavelength for mercury can be seen in Fig.3.

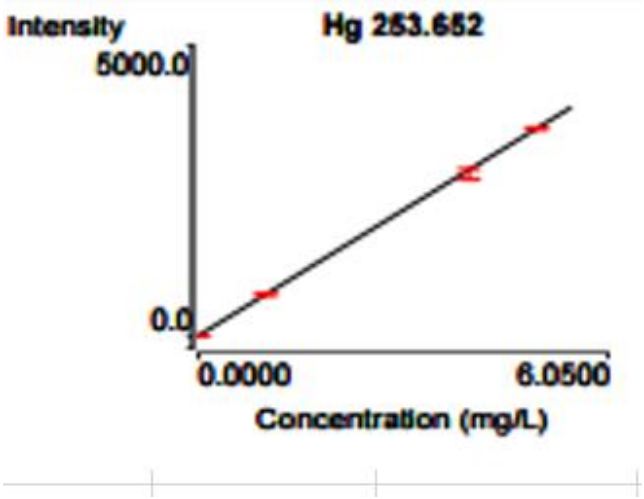

Figure 3. Determination of Maximum Wavelength for Mercury

There are 9 samples indicated to contain mercury with varying concentrations. The calculated mercury concentrations in the samples can be seen in Table 4.

Table 4. Mercury contain

No	Sample Code	Concentration (mg/L)
1	A	-0.5902
2	B	-0.5989
3	C	-0.2188
4	D	-0.0092
5	E	-0.0071
6	F	-0.0069
7	G	-0.5997
8	H	-0.6018
9	I	138.4844

Samples A to H have concentrations ranging from -0.0071 to -0.6018 mg/L. When converted to mg/kg, the mercury concentration in these samples is less than 0.0001. On the other hand, sample I has a high concentration of 138.4844 mg/L. The use of mercury in beauty products such as facial cleansers, moisturizers, day creams, and night creams is strictly prohibited due to its severe health risks, including kidney disorders, permanent brain damage, and fetal abnormalities. The permissible limit for mercury content in eye makeup and its removers is still allowed, but it should not exceed 0.007%.

CONCLUSION

Based on the results of the conducted research, it can be concluded that the qualitative test for mercury using KI reagent yielded positive indications for the presence of hydroquinone in the

whitening cream samples with codes A, B, C, D, F, and I, while the samples with codes E, G, H tested negative.

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