DETERMINATION OF RETINOIC ACID LEVELS IN WHITENING CREAM PREPARATIONS CIRCULATING IN THE KUDUS CITY

Yanulia Handayani [1], Susan Primadevi[2], Agathi Valentina[3]

Health Technology Institute Cendekia Utama Kudus, Indonesia

*Corresponding Author: yanulia.handayani@gmail.com

Abstract. Retinoic acid is one of the active substances found in cosmetics. This study aims to determine the levels of retinoic acid in whitening cream preparations and compare the levels of retinoic acid with safe limits according to BPOM. To find out whether the whitening cream circulating in the Kudus City contains retinoic acid find out how much retinoic acid is in the whitening cream and find out whether the whitening cream circulating in the Kudus City is safe to use according to BPOM standards. In this study, the Thin Layer Chromatography method was used for qualitative analysis with n-hexane eluent: acetone (6:4) v/v. The results were observed under UV254 nm light. The UV-Vis spectrophotometric method was used for quantitative analysis with a wavelength of 288 nm. The TLC test results for samples A, B, C, D, and H had Rf values and spot colors that were almost the same as the reference standard for retinoic acid. The Rf values for samples A, B, C, D, and H were 0.6, 0.63, 0.6, 0.6, and 0.57, respectively. Meanwhile, the standard Rf for retinoic acid is 0.6. Retinoic acid levels obtained from samples A, B, C, D, and H were 0.2769%, 0.1209%, 0.1205%, 0.2756%, and 0.2809% respectively. Samples A, B, C, D, and H were positive for retinoic acid, with the levels of retinoic acid obtained still within safe limits according to BPOM.

Keywords: [Retinoic acid, Bleaching cream, Thin Layer Chromatography, UV-Vis Spectrophotometry]

INTRODUCTION

Beautiful and youthful are two things that women always dream of. They do various ways to get these two things (Firmansyah, 2013). One way that is often done is to use cosmetics. There are many choices of cosmetic products used by women to make them look more beautiful, including whitening creams. Face whitening creams are useful for faces that have various problems on the face because they can restore brightness to the skin and reduce the black color of the face (Erasiska, Bali & Hanifah, 2015). Some whitening creams contain active ingredients which vary depending on the purpose for which they are used. The Food and Drug Administration (BPOM) has issued a public warning or warning that several brands of facial care cosmetics, especially day and night creams contain dangerous ingredients. One of these dangerous ingredients is retinoic acid (Rahayu, Nurulita & Septianingrum, 2014). Retinoic acid is a type of chemical compound related to vitamin A. Retinoic acid has a low molecular weight, which has a biological effect on vision, tissue repair, cell growth, differentiation of various epithelia in the body, facilitates immunomodulation action, and helps cell change, despite having many benefits, retinoic acid with wrong use can cause contraindications (Nursidika, Sugihartina & Fransiska, 2018). The side effects of using retinoic acid are local irritation at the beginning of therapy, namely erythema, burning, flaky and dry skin/xerosis. Higher concentrations predispose to more severe irritation. Retinoic acid causes more irritation when used by patients with eczema, rosacea, or other skin sensitivity disorders (Fauzia, 2017). Recognizing that retinoic acid is harmful to consumers, a study will be carried out to determine the levels of retinoic acid in whitening creams by Thin Layer Chromatography and UV-Vis Spectrophotometry.

METHODS

Material

The materials used were methanol, acetone pa, n-hexane pa, retinoic acid, and a sample of whitening cream from Kota Kudus.

Tool

The tools used were Erlenmeyer, beaker, measuring flask, funnel, volume pipette, dropping pipette, capillary tube, stir bar, Whatman filter paper no.41, aluminum foil, analytical balance, UV254 lamp, chromatography vessel, silica gel 60F254, UV-Vis spectrophotometer.

Preparation of sample test solutions containing retinoic acid
A total of 3 grams of sample was weighed and put into a beaker, then wrapped in aluminum foil. Into the beaker added 10 mL of methanol and then shake until homogeneous. The solution was cooled in ice for 15 minutes, then filtered using Whatman paper no. 41.

Development of Developer Solutions
The n-hexane–acetone solution (6:4) was put into the chamber then covered with a glass plate and left to stand until the eluent was saturated.

Sample identification with Thin Layer Chromatography
The KLT plate was activated by heating it in an oven at 105°C for 30 minutes by making a spotting limit and an elution limit of 7 cm. The test solution was bottled separately using a capillary tube at a distance of 1.5 cm from the bottom of the plate. Then leave it for a while to dry. The KLT plate which already contains the sample is put into the KLT vessel which is first saturated with the mobile phase in the form of n-hexane and acetone (6:4). Let the phase move up until it approaches the elution limit. Then the KLT plate was removed and allowed to air dry. Observed under fluorescent UV 254nm light giving dark spots.

Quantitative analysis of retinoic acid using UV spectrophotometry
Preparation of standard retinoic acid solution
Concentration 1000 ppm
Retinoic acid was weighed as much as 0.1 gram, then put into a beaker, dissolved and diluted with 100 mL of methanol, and homogenized.
Concentration 100 ppm
Take 1 mL of a standard 1000 ppm retinoic acid solution, then put it into a 10 mL volumetric flask, and add methanol until the mark is homogenized.

Determination of the maximum wavelength of retinoic acid
Take 2 mL of 100 ppm retinoic acid solution and put it in a volumetric flask 10 mL (20 ppm concentration). Add methanol up to the limit line and homogenize. The maximum absorption was measured at a wavelength of 288 nm using a blank. The blank used methanol.

Determination (Operating Time)
Take 2 mL of 100 ppm retinoic acid solution and put it in a volumetric flask 10 mL (20 ppm concentration). Add methanol up to the boundary line and homogenize. Then read the absorbance at 0-60 minutes at the maximum wavelength.

Preparation of standard solutions
Pipette 100 ppm of retinoic acid solution and put it in a 10 mL measuring flask. 0.25 mL, 0.5 mL, 1.0 mL, 1.25 mL, 1.5 mL, and 2.0 mL (12.5 ppm, respectively) 25 ppm, 50 ppm, 62.5 ppm, 75 ppm, and 100 ppm). Then added methanol until the boundary marks were shaken until dissolved, then the absorption was measured at the maximum wavelength obtained using a blank solution.

Quantitative test on the sample
A total of 3 grams of the test sample was weighed and put into a beaker, then wrapped in aluminum foil. Added 10 mL of methanol and shake until homogeneous. Cooled on ice for 15 minutes and filtered using Whatman filter paper no.41. Then pipette 1 mL into a 10 mL volumetric flask. Methanol was added up to the mark and homogenized. Then the absorption is measured at the maximum wavelength.

Data analysis
Data that has been obtained through the spectrophotometric method will present the results based on the calibration curve with the linear regression equation $y = a + bx$ where:
Y: absorbance
X: concentration
b: coefficient (slope = slope)

a: regression constant (intercept)
The research sample will get 3 treatments. In this study, data analysis was presented in the form of tables, diagrams, and calibration curves and then narrated and conclusions were drawn.

RESULTS AND DISCUSSION

Results of Retinoic Acid Qualitative Analysis with Thin Layer Chromatography Method

From the elution results on thin layer chromatography, it is known that samples A, B, C, D, and H are positive for retinoic acid because they have an Rf value that is almost the same as the reference standard Rf of 0.6. The Rf values of samples A, B, C, D, and H were 0.6, 0.63, 0.6, 0.6, and 0.57, respectively. The color of the stain between the reference standard and the positive sample is also the same, namely yielding dark spot color. Meanwhile, samples E, F, G, I, and J were declared negative with Rf values of 0.9, 0.86, 0.84, 0.88, and 0.88, respectively. Samples E and G have purple stains. Samples F, I, and J had slightly darker spots but the Rf values were far from the reference standard, meaning that these samples were negative for retinoic acid. Compounds on Thin Layer Chromatography are said to be identical if they have the same stains and Rf values as the reference compound. The results can be seen in Table 1.

Table 1. Retinoic Acid Qualitative Test on Samples by KLT Method

Sample	Rf value	Stain Color	Results
A	0.6	Dark patches	+
B	0.63	Dark patches	+
C	0.6	Dark patches	+
D	0.6	Dark patches	+
E	0.9	Purple blotches	-
F	0.86	Dark patches	-
G	0.84	Purple blotches	-
H	0.57	Dark patches	+
I	0.88	Dark patches	-
J	0.88	Dark patches	-
Comparison Standard	0.6	Dark patches	+

Results of Quantitative Analysis of Retinoic Acid with UV Vis Spectrophotometry Method

Maximum Wavelength Determination

From the results of the experiments conducted, the maximum wavelength with a concentration of 20 ppm is 288 nm. The maximum wavelength chosen for the determination of retinoic acid levels in this study was 288 nm. This wavelength was chosen because it is at its theoretical maximum wavelength, which is between 200-400 nm. Presented in table 2.

Table 2. Maximum Wavelength Result

Maximum Wavelength (nm)	absorbance
285.0	0.233
286.0	0.268
287.0	0.307
288.0	0.331
289.0	0.317
290.0	0.298
291.0	0.280

Setting Operating Time

The research was carried out by mixing a standard solution of retinoic acid with methanol as a solvent. Measurement with a concentration of 20 ppm at a maximum wavelength of 288 nm. The

absorbance formed was measured for 60 minutes. From the operating time measurement spectrum, it can be seen that the absorbance produced has been stable since the 2nd minute. This is indicated by the spectrum that forms an almost straight line at the 2nd minute, meaning that in that time span, the absorbance of the compounds measured is relatively stable. presented in Table 3 and Figure 1.

Table 3. Operating time results

Time (minutes)	absorbance
0	0.387
1	0.469
2	0.360
3	0.360
4	0.360
5	0.376
6	0.428

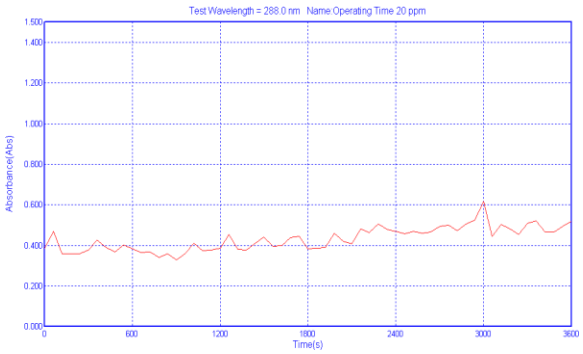

Figure 1. Setting the Operating Time

Determination of the Standard Curve

In this study, 6 series of standards were used with different concentrations, namely 12.5 ppm, 25 ppm, 50 ppm, 62.5 ppm, 75 ppm, and 100 ppm, which were measured at a wavelength of 288 nm. Based on the standard curve, a linear regression equation is obtained y = 0.0075x + 0.0361 and an R-value of 0.9844. It can be seen in table 4 and the standard curve diagram can be seen in figure 2.

Table 4. Standard Curve Data

Retinoic Acid Raw Series Concentration (ppm)	absorbance
12.5	0.117
25	0.229
50	0.455
62.5	0.542
75	0.623
100	0.754

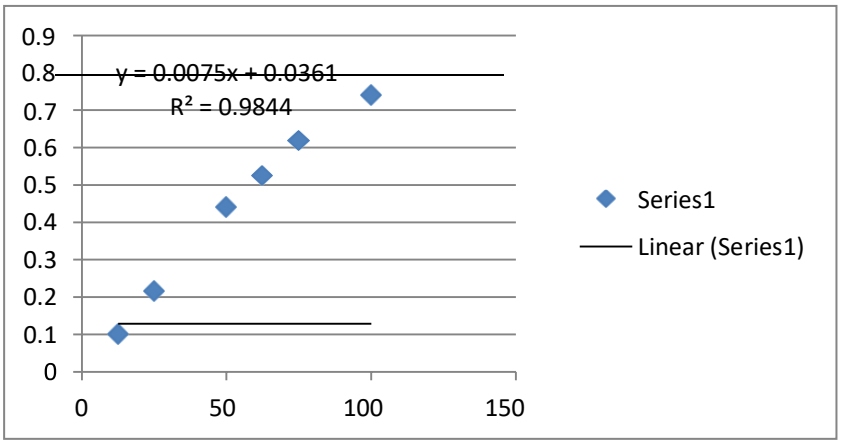

Figure 2. Retinoic Acid Standard Curve

Sample levels

From the research conducted, it was found that samples A, B, C, D, and H obtained retinoic acid levels of 0.2769%, 0.1209%, 0.1205%, 0.2756%, and 0.2809%, respectively. The highest level of retinoic acid was found in sample B while the lowest level was found in sample C, which was 0.1205%. The results of determining the levels of positive samples containing retinoic acid can be seen in Table 5.

Table 5. Retinoic Acid Levels

Sample	Average absorbance	Average Standard Deviation	Sample Rate
A	0.587	0.004359	0.2769 %
B	0.236	0.008021	0.1209 %
C	0.235	0.013317	0.1205 %
D	0.584	0.010017	0.2756 %
H	0.596	0.029206	0.2809 %

Of the 5 cream samples that positively contained retinoic acid, it could be concluded that the retinoic acid content was still within the specified safe limits, namely 0.001-0.4%.

CONCLUSION

From the results of this study, it can be concluded that the results of the Thin Layer Chromatography test showed that several face-whitening creams circulating in Kudus City were positive for containing retinoic acid, namely creams A, B, C, D, and H. Retinoic acid levels in samples A, B, C, D, and H were 0.2769%, 0.1209%, 0.1205%, 0.2756% and 0.2809%, respectively.

ACKNOWLEDGEMENT

From the results of this study, it is suggested that the sampling carried out is not extensive so it does not represent the expected population. The safety parameters of the whitening cream tested were only retinoic acid, so they could not represent the overall safety parameters of the whitening cream.

REFERENCES

Afifah, H., (2015). Analisis Kualitatif dan Kuantitatif Asam Retinoat dan Uji Kualitatif Merkuri pada sediaan krim pemutih yang beredar dibeberapa tempat di Kota Bandung. Skripsi. *Program Sarjana Farmasi Fakultas Matematika dan IPA*, Universitas Al- Ghifari, Bandung.

Andriyani, Vina B., (2011). Identifikasi Asam Retinoat dalam krim pemutih wajah secara Kromatografi Lapis

Tipis. Tugas Akhir. Program Diploma 3 Analisis Farmasi dan Makanan, Fakultas Farmasi, Medan.

Armin, F., Zulharmita & Firda, (2013). 'Identifikasi dan penetapan kadar merkuri'. Jurnal Sains dan Teknologi Farmasi, Vol. 18 (1), p. 28-34.

Badan POM RI, (2007). Public Warning / Peringatan tentang kosmetik mengandung bahan berbahaya dan zat warna yang dilarang. Jakarta.

Badan POM, (2011). Mewaspadai Asam Retinoat dalam Kosmetik. Jakarta

Erasiska, Bali & Hanifah, (2015). "Analysis of the content of lead, cadmium, and mercury in facial whitening cream products". Online Journal of Students of the Faculty of Mathematics and Natural Sciences, Vol. 2 (1), p.123-129.

Fauzia, D., (2017). 'Pharmacological aspects of retinoate in cosmeceuticals'. Malay Health Journal, p. 35-40.

Firmansyah, (2013). 'The tragedy of the bloody beauty of Elizabeth Bathory: A story with illustrations'. Article. Visual Communication Design Study Program, Bandung.

Nursidika, P., Sugihartina, G., & Fransiska, I., (2018). 'Retinoic acid in whitening creams sold online'. Journal of Proceedings of the National Scientific Meeting for Research and Community Service, Vol. 1(1), p. 622-626.

Rahayu, Nurulita & Septianingrum, (2014). 'Analysis of retinoic acid in over-the-counter whitening cream preparations in Purwokerto'. Journal of Proceedings of the National Seminar and Workshop "Recent Developments in Pharmaceutical & Clinical Science IV". Faculty of Pharmacy Muhammadiyah University Purwokerto, p. 1- 6.