

Determination Method of Retinoic Acid Using High Performance Liquid Chromatography and Application on the Whitening Night Cream

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Abstrak-Asam retinoat secara umum telah digunakan untuk pengelupas kulit dalam perawatan pemutihan wajah. Konsentrasi asam retinoat pada sediaan kosmetik topikal berdasarkan persyaratan adalah 0,025-0,1% sehingga memerlukan metode yang tervalidasi untuk menentukan kadarnya. Tujuan dari penelitian ini adalah untuk memvalidasi metode penetapan kadar asam retinoat menggunakan KCKT dan menerapkan metode tervalidasi tersebut pada krim malam pemutih wajah. Penetapan kadar asam retinoat menggunakan KCKT (Jasco PU-2080 plus) yang dilengkapi detektor ultraviolet (2070 plus) pada panjang gelombang 340,3 nm. Fase diam yang digunakan adalah C18 (12,5 cm x 4 mm) dan fase gerak berupa campuran metanol:air:asam asetat glasial (90:10:0,5, v/v/v), dengan laju alir 1,3 mL/menit. Parameter validasi meliputi presisi, akurasi, linearitas, sensitivitas (LOD dan LOQ) dan selektivitas. Sampel diambil dari klinik kecantikan di Semarang. Metode analisis yang diusulkan telah memenuhi persyaratan validasi. Uji presisi menghasilkan nilai RSD 0,09% -0,37%, uji akurasi memberikan nilai rekoveri untuk sampel A adalah 99,92% -100,78% dan sampel B adalah 99,44% -101,42%, linieritas yang dihasilkan baik dengan koefisien korelasi (r) = 0,9996, LOD dan LOQ masing-masing adalah 0,16 $\mu\text{g/mL}$ dan 0,54 $\mu\text{g/mL}$ dan metode memiliki selektivitas yang baik. Kandungan asam retinoat dalam sampel A adalah 0,02% dan sampel B adalah 0,02%. Hasil penelitian menunjukkan bahwa konsentrasi asam retinoat dalam sampel tidak berlebihan.

Kata kunci: metode penetapan kadar, asam retinoat, krim malam pemutih wajah, HPLC

Abstract-Retinoic acid is commonly used for bleaching facial in the whitening treatment. The concentration of retinoic acid in a topical preparation is 0.025 to 0.1% based on the cosmetics requirements so that it needs a validated determination method. The aims of this study were to validate the determination method of retinoic acid using HPLC and to apply the validated method on the whitening night cream. Determination of retinoic acid using a HPLC (Jasco PU-2080 plus) equipped with ultraviolet detector (2070 plus) at a wavelength of 340.3 nm. The stationary phase used C18 (12.5 cm x 4 mm) and the mobile phase used a mixture of methanol:water:glacial acetic acid (90:10:0.5, v/v/v), delivered with flow rate of 1.3 mL/min. The method was validated in parameter terms of precision, accuracy, linearity, sensitivity (LOD and LOQ) and selectivity. The applied samples were taken from beauty clinics in Semarang. The proposed method complied with the validity requirements. RSD value of precision test was 0.09%-0.37%, accuracy test resulted recovery value for sample A was 99.92%-100.78% and sample B was 99.44%-101.42%, good linearity with a correlation coefficient (r) = 0.9996, LOD and LOQ were 0.16 $\mu\text{g/mL}$ and 0.54 $\mu\text{g/mL}$, respectively and the method had a good selectivity. Retinoic acid in sample A was 0.02% and sample B was 0.02%. The results showed that the concentration of retinoic acid in the samples were not excessive.

Keywords: determination method, retinoic acid, whitening night cream, HPLC

1. Introduction

Retinoic acid is commonly used for bleaching facial in the whitening treatments and used for repairing skin from sundamage. Retinoic acid causes dangerous risks include skin inflammation and skin hardening. Potential as a carcinogen has been proven in albino mice and pigmented mice that were

exposed with UV-A and UV-B (National Toxicology Program, 2012). Retinoic acid can cause defects in the fetus due to the effect as a teratogen substances (Puspitadewi, 2008).

Determination of retinoic acid in the cosmetic dosage forms requires a appropriate analytical methods for quality control purposes. Several methods have been developed to determine retinoic acid such as High Performance Liquid Chromatography (HPLC) with UV detector. Retinoic acid has an aromatic ring, conjugated double bonds and auxochrome anion-O therefore can be detected by a UV detector (Nastiti, 2016).

Analysis method of retinoic acid in the bleaching creams market has been validated by Rahayu (2014) with a mobile phase consisted of methanol:water:glacial acetic acid (85:15:0.5, v/v/v), C18 column and wave length setted at 353 nm. The results met the parameters requirement of linearity, LOD and LOQ.

Nastiti (2016) conducted an analysis of retinoic acid in whitening creams using HPLC. A mobile phase consisted of methanol:water:glacial acetic acid (90:10:0.5, v/v/v), the stationary phase C18 column, with a UV detector setted at 353 nm. The method has been validated on precision, linearity, LOD and LOQ.

The concentration of retinoic acid in a topical preparation is 0.025 to 0.1% based on the cosmetics requirements, so it needs a very sensitive method (Draelos and Thaman, 2006). However, there was a few report regarding the determination method of retinoic acid in whitening night cream. The aims of this study were to validate the determination method of retinoic acid using HPLC and to apply the validated method on the whitening night cream.

2. Research Method

2.1. Materials

Reference standards retinoic acid (Multi Kimia Raya), aquabidestilata (PT. Ikapharmindo), HPLC grade methanol (Merck), glacial acetic acid (Merck), samples of a whitening night cream from beauty clinic A and B.

2.2. Tool

A set of HPLC (Jasco PU-2080 plus) equipped with ultraviolet detector (2070 plus), C18 column 12.5cmx4mm, diameter of 5 μ m. UV-Vis spectrophotometer (Shimadzu UV-1800), sentrifugator (Hettich EBA 20), vortex mixer (H-VM-300), an ultrasonic bath (Jeken), 0.45 μ m membrane filter, vacuum degasser pump (Rocker 300), scale analytic (Pioneer Ohaus PA214), micro pipette size 5-50 mL and 100-1000 mL (Soccorex).

2.3. Preparation of Stock and Working Solutions

A stock solution of retinoic acid with the concentration of 400 μ g/mL was prepared by dissolving 10.0 mg retinoic acid into 25 mL volumetric flask and diluted with methanol. The working solution was obtained by appropriate dilution to give the concentration of substance at 2, 3, 4, 5 and 6 μ g/mL.

2.4. Chromatography

Determination was performed on reversed phase at ambient temperature. The optimization of mobile phase was carried out by varying methanol:water:acetic glacial acid with ratio of (80:20:0.5, v/v/v; 85:15:0.5 v/v/v and 90:10:0.5, v/v/v). Flow rate was optimized at 1.0; 1.3 and 1.4 mL/minute. The UV detection was set at 340.3 nm. The injection volume was 20 μ L.

2.5. Analytical Method Validation

The developed HPLC method was validated according to guideline in International Conference Harmonization (2005) by assessing several parameters namely linearity, sensitivity expressed with limit of detection (LOD) and limit of quantification (LOQ), precision and accuracy.

2.6. Preparation

A sample night cream was weighed 1,0 gram and then diluted with 10 ml of methanol and mixed using a vortex mixer for 5 minutes. Centrifugation with a speed of 600 rpm for 10 minutes then filtered with a membrane filter of 0.45 μL . A sample was diluted 5 times by taking 1 mL of solution into a 5 mL flask. The solution was filtered using membrane filter 0.20 μm and 20 μL of filtered solution was injected into the HPLC system. There were six repetitions for each sample a night cream. Quantification of retinoic acid was performed using an external standard technique.

3. Result and Discussion

3.1. Optimization of Mobile Phase Composition

Optimization was carried in order to get certain polarity which increases the selectivity and sensitivity of the analytical method. Retinoic acid is easily soluble in methanol, a polar solvent that is usually used in reversed phase chromatography. Aquabidestilata was used to adjust the polarity of mobile phase. Glacial acetic acid was used to control the acidity due to ionization of analytes, besides as a modifier column of silica to prevent and to reduce the tailing peak (Munson, 1991). Figure 1 shows that the mobile phase consisting of methanol:water:glacial acetic acid (90:10:0.5, v/v/v) produced retention time at 5.36 minute and good shape chromatogram peak.

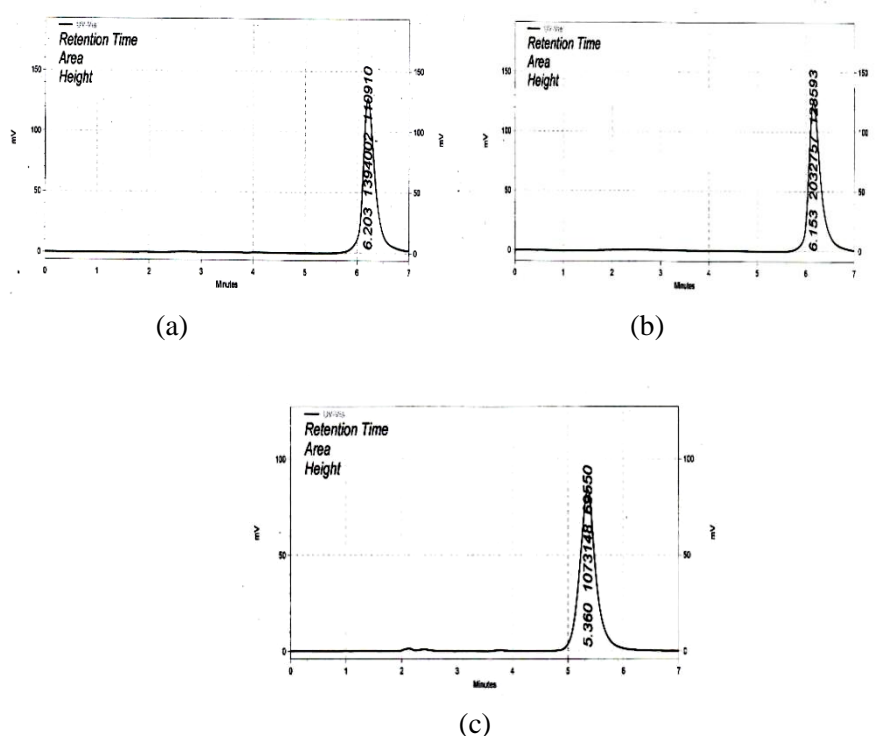


Figure 1. Optimization of Mobile Phase

(a) of methanol: water: glacial acetic acid (80:20:0.5, v/v/v)

(b) of methanol: water: glacial acetic acid (85:15:0.5, v/v/v)

(c) of methanol: water: glacial acetic acid (90:10:0.5, v/v/v)

3.2. Standard Working Curve of Retinoic Acid

The equation of linier regression is $y=184462.48x-111.456$. The equation obtained was used to determine retinoic acid in the samples.

3.3. Validation of Analysis Method

3.3.1. Precision

Precision test used retinoic acid solution at 3 µg/mL. Replicated six times. RSD value of 0.09% - 0.37%, smaller than the requirement, 2%.

3.3.2. Accuracy

Accuracy test carried by standard additions method. Test conducted with spiking raw material into samples at 80%, 100%, 120%. Recovery values are in the accepted range 98-102% (Gandjar and Rohman, 2012). The analytical method can be used to produce data that close or similar to the appropriate concentration.

3.3.3. Linierity

The regression linear based on concentration against absorbance was $y=184462.48x-111.456$ with correlation factor (r) = 0.9996 (figure 2).

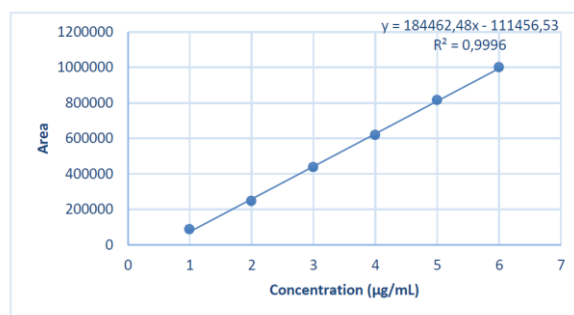


Figure 2. Regression Linear of Retinoic Acid Working Solutions

3.3.4. Sensitivity

Sensitivity parameter of analysis method is represented by limit of detection (LOD) and limit of quantification (LOQ). Based on the calculation using regression linier, LOD value of 0.16 µg/mL and LOQ value of 0.54 µg/mL.

3.3.5. Selectivity

Selectivity of the analysis method is represented by Resolution value (R). The HPLC analysis method has a good selectivity with no disturbance from other component.

3.4. Determination of Retinoic Acid in the Whitening Night Cream

Based on the parameters values, it was stated that the proposed method complied with the validity requirements and appropriate to be used for determining retinoic acid in the samples. Retinoic acid in sample A was 0.02% and sample B was 0.02%. The results showed that the concentration of retinoic acid in the samples were not excessive.

4. Conclusion

It can be concluded that analytical method using HPLC at a wavelength of 340.3 nm with the stationary phase used C18 and a mobile phase consisting of methanol:water:glacial acetic acid (90:10:0.5 v/v/v) and flow rate of 1.3 mL/minute allows quantitative analysis of retinoic acid and the analytical method was valid to be applied for whitening night cream were taken from beauty clinics in Semarang and the concentration of retinoic acid in the samples were not excessive.

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