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Method of Test for Hydroquinone, Hydroquinone Monobenzyl Ether, Rhododendrol and Tretinoin in Cosmetics

1. Scope

This method is applicable to the identification and determination of hydroquinone, hydroquinone monobenzyl ether, rhododendrol, and tretinoin in cosmetics.

2. Method

After extraction, analytes are determinated by high performance liquid chromatography (HPLC).

2.1. Equipments

2.1.1. High performance liquid chromatograph.

2.1.1.1. Detector: photodiode array detector.

2.1.1.2. Column: CAPCELL PAK C18 UG, 5 μm, 4.6 mm i.d. × 250 mm; or an equivalent product.

2.1.2. Ultrasonicator.

2.2. Chemicals

Methanol, HPLC grade;
Acetonitrile, HPLC grade;
Formic acid, GR grade;
Deionized water, resistivity ≥ 18 MΩ•cm (at 25^{o}C);
Hydroquinone, hydroquinone monobenzyl ether, rhododendrol and tretinoin, reference standards.

2.3. Apparatus

2.3.1. Volumetric flask: 10 mL, 20 mL, 50 mL and 100 mL.

2.3.2. Membrane filter: 0.45 μm, Nylon.

2.4. Standard solution preparation

Accurately weigh equivalent 5 mg of hydroquinone, hydroquinone monobenzyl ether, rhododendrol, and tretinoin to each 10-mL volumetric flask, dissolve and dilute with methanol to volume as standard stock solutions. When to use, mix appropriate volume of each standard stock solution and dilute with methanol to 10-500 μg/mL for hydroquinone, hydroquinone monobenzyl ether and rhododendrol, and 0.5-50 μg/mL for tretinoin respectively as the standard

solutions.

2.5. Sample solution preparation

Transfer about 1 g of the well-mixed sample accurately weighed into a 10-mL volumetric flask, add 8 mL of methanol, and ultrasonicate for 30 min. Dilute to volume with methanol and filter with a membrane filter. Take the filtrate as the sample solutions.

2.6. Identification and Quantitation

Accurately inject 20 µL of the sample solution and the standard solutions into the HPLC separately, and operate according to the following conditions. Identify each analyte based on the retention time and the UV absorption spectrum. Calculate the amount of hydroquinone, hydroquinone monobenzyl ether, rhododendrol, and tretinoin in the sample by the following formula:

The amount of hydroquinone, hydroquinone monobenzyl ether, rhododendrol, and tretinoin in the sample (ppm) = $\frac{C \times V}{M}$

Where,

C: the concentration of each analyte in the sample solution calculated by its standard curve (µg/mL)

V: the make up volume of sample (mL)

M: the weight of sample (g)

HPLC operating conditions:

Photodiode array detector: the quantitative wavelength for each analyte is as follows.

Analytes	Wavelength (nm)
Hydroquinone Hydroquinone monobenzyl ether Rhododendrol	280
Tretinoin	350

Column: CAPCELL PAK C18 UG, 5 µm, 4.6 mm i.d. × 250 mm.

Injection volume: 20 µL.

Mobile phase: a gradient program of solvent A (deionized water) and solvent B (acetonitrile) is as follows.

Time (min)	Solvent A (%)	Solvent B (%)
0.0 → 1.0	85 → 85	15 → 15
1.0 → 2.0	85 → 50	15 → 50
2.0 → 6.0	50 → 50	50 → 50
6.0 → 7.0	50 → 5	50 → 95
7.0 → 16.0	5 → 5	95 → 95
16.0 → 18.0	5 → 85	95 → 15
18.0 → 20.0	85 → 85	15 → 15

Flow rate: 1 mL/min

Note: All the parameters can be adjusted depending on the instruments used if the above conditions are not applicable.

Remark

1. The limits of quantitation (LOQs) are as follows: 10 ppm for hydroquinone, hydroquinone monobenzyl ether and rhododendrol, and 5 ppm for tretinoin.
2. Further validation shall be done when interference compounds appear in samples.

Reference

1. Tashtoush, B. M., Jacobson, E. L. and Jacobson, M. K. 2007. A rapid HPLC method for simultaneous determination of tretinoin and isotretinoin in dermatological formulations. J. Pharm. Biomed. Anal. 43: 859-864.
2. Rychlinska, I. and Nowak, S. 2012. Quantitative determination of arbutin and hydroquinone in different plant materials by HPLC. Not. Bot. Horti. Agrobo. 40: 109-113.

Reference chromatogram

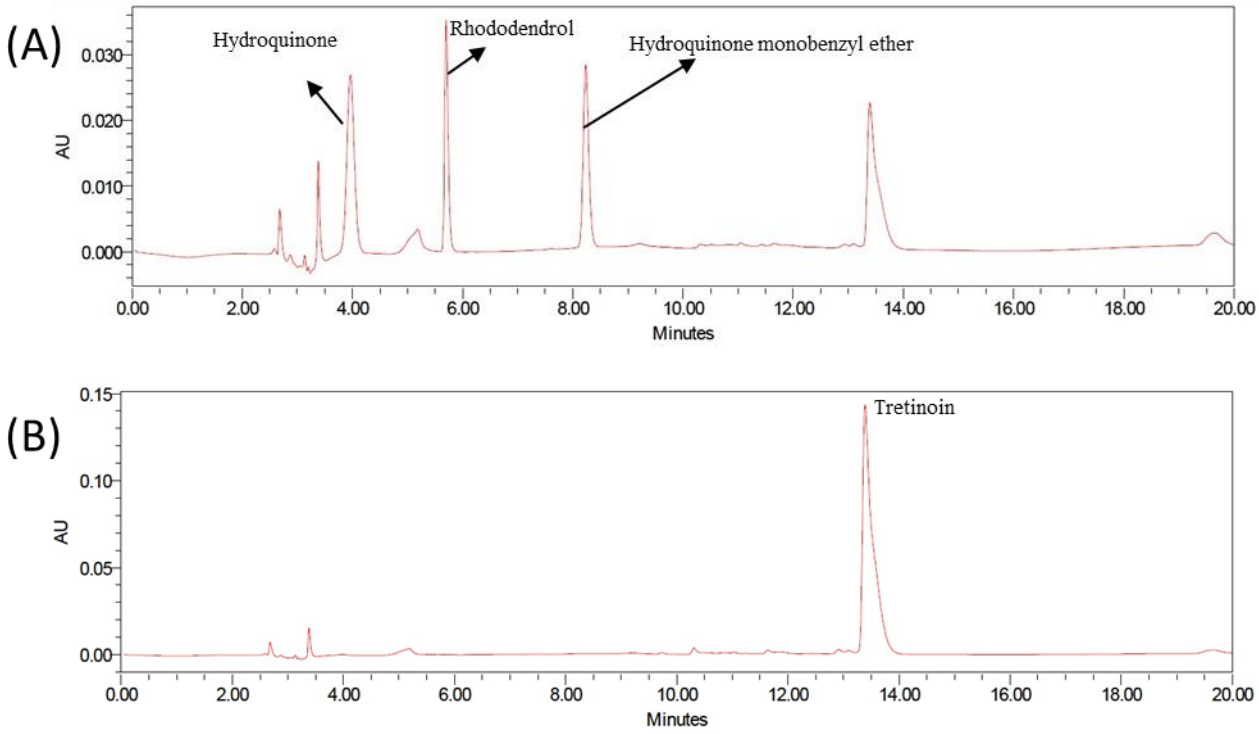

Figure. The HPLC chromatograms of hydroquinone, hydroquinone monobenzyl ether, rhododendrol, and tretinoin standards detected at the wavelength of 280 nm (A) and 350 nm (B).