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**Determination of prohibited urocanic acid and its ethyl
ester in cosmetics - High performance liquid
chromatography**

化妆品中禁用物质尿刊酸及其乙酯的测定 高效液相色谱法

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Annex A (informative) Chinese name, English name, CAS number, molecular
formula, relative molecular mass, structural formula of urocanic acid and its
ethyl ester **Error! Bookmark not defined.**

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Determination of prohibited urocanic acid and its ethyl ester in cosmetics - High performance liquid chromatography

1 Scope

This Standard specifies determination method of high performance liquid chromatography for prohibited urocanic acid and its ethyl ester in cosmetics.

This Standard is applicable to determination of urocanic acid and its ethyl ester in cosmetics such as creams, liquids, lipsticks, loose powders, shampoos.

The detection limit of this Standard for urocanic acid and its ethyl ester is 2 mg/kg, and the limit of quantification is 5 mg/kg.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

GB/T 6682, *Water for analytical laboratory use - Specification and test methods*

3 Principle

After the specimen is centrifuged by solvent and is centrifuged, after the supernatant is filtered by nitrogen blowing capacity, use high performance liquid chromatography to determine, use external standard method to quantify, use liquid chromatography-mass spectrometry to confirm.

4 Reagents and materials

Unless otherwise stated, the reagents used are analytically pure, the water is of grade one specified in GB/T 6682.

4.1 Methanol: chromatographically pure.

5.5 Ultrasonic cleaner.

5.6 Nitrogen blower.

5.7 Plugged plastic centrifuge tube: 10mL.

5.8 Graduated nitrogen blow tube: 10mL.

5.9 Microporous membrane: 0.45μm, made of PTFE.

6 Analysis steps

6.1 Sample processing

6.1.1 Cream, liquid, loose powder and shampoo samples

Weigh 0.2g (to the nearest of 0.001g) of specimen in a 10mL plugged plastic centrifuge tube (5.7). Add into 10mL of methanol (4.1). Shake well. After performing ultrasonic extraction for 20min, centrifugation at 8000r/min for 15min, transfer the supernatant to the graduated nitrogen blow tube (5.8). Slow the nitrogen blowing to 1mL. Add into 9mL of water. Mix well. It is filtered through a 0.45μm microporous membrane for high performance liquid chromatography.

6.1.2 Lipstick sample

Weigh 0.2g (to the nearest of 0.001g) of specimen in a 10mL plugged plastic centrifuge tube (5.7). Add into 0.5mL of tetrahydrofuran (4.2). Then add into 9.5mL of methanol (4.1). Shake well. Performing ultrasonic extraction for 20min, centrifugation at 8000r/min for 15min. Transfer the supernatant to the graduated nitrogen blow tube (5.8). Slow the nitrogen blowing to 1mL. Add into 9mL of water. Mix well. It is filtered through a 0.45μm microporous membrane for high performance liquid chromatography.

6.2 Determination conditions

The reference conditions for high performance liquid chromatography are as follows:

- a) Chromatographic column: Xterra MS C₁₈, 5μm, 250mm × 4.6mm (inner diameter), or the equivalent;
- b) Flow rate: 1.0mL/min;
- c) Mobile phase: methanol + 0.005mol/L tetrabutylammonium bromide solution=30+70 (volume ratio);
- d) Detection wavelength: 285nm;

Annex C

(informative)

Test of confirmation

C.1 Liquid chromatography conditions

The reference conditions for liquid chromatography determination are as follows:

- a) Chromatographic column: XBridge C₁₈, 3.5μm, 150mm × 2.1mm (inner diameter);
- b) Mobile phase: 0.1% formic acid solution + acetonitrile = 95 + 5 (volume ratio);
- c) Flow rate: 0.3mL/min;
- d) Column temperature: 30°C;
- e) Injection volume: 5μL.

C.2 Mass spectrometry conditions

The reference conditions for mass spectrometry are as follows:

- a) Ion source: electrospray ion source;
- b) Ionization mode: positive ion mode;
- c) Capillary voltage: 3kV;
- d) Extraction voltage: 2.9V;
- e) Ion source temperature: 150°C;
- f) Desolvation gas temperature: 350°C;
- g) Data collection method: multiple reaction monitoring.

C.3 Qualitative determination

When the specimen is measured, the sample solution is appropriately diluted, and the sample solution and the standard working solution are determined according to liquid chromatography-mass spectrometry conditions. If the selected ions are present, and the selected ion ratio is consistent with the

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