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Identification and Assay for Allantoin and Urea in Cosmetics

1. Scope

This method is applicable to the determination of allantoin and urea in cosmetics.

2. Method

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

2.1. Equipment

2.1.1. High performance liquid chromatograph.

2.1.1.1. Detector: photodiode array detector.

2.1.1.2. Column: COSMOSIL-HILIC, 5 μm, 4.6 mm i.d. × 25 cm, or an equivalent product.

2.1.2. Ultrasonicator.

2.2. Chemicals

Acetonitrile, HPLC grade;
Deionized water, resistivity ≧ 18 MΩ·cm (at 25°C);
Allantoin and Urea, reference standard.

2.3. Apparatus

2.3.1. Volumetric flask: 10 mL, 20 mL.

2.3.2. Membrane filter: 0.45 μm, Nylon.

2.4. Mobile phase

Mix acetonitrile and deionized water at the ratio of 93:7 (v/v), and filter with a membrane filter.

2.5. Standard solution preparation

Transfer about 10 mg of allantoin and 200 mg of urea reference standards accurately weighed into each 10-mL volumetric flask, dissolve and dilute with deionized water to volume as the standard stock solutions. When to use, mix appropriate volume of each standard stock solution and dilute with deionized water to 5-100 μg/mL for allantoin, 250-5000 μg/mL for urea as the standard solutions.

2.6. Sample solution preparation

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

2.7. Identification and quantitation

Accurately inject 10 µL of the sample solution and the standard solutions into HPLC separately, and operate according to the following conditions. Identify allantoin and urea based on the retention time and the absorption spectrum. Calculate the amount of allantoin or urea in the sample by the following formula:

$$\text{The amount of allantoin or urea in the sample (\%)} = \frac{C \times V}{M} \times 10^{-4}$$

where,

C: the concentration of allantoin or urea in the sample solution calculated by the standard curve (µg/mL)

V: the final make-up volume of the sample (mL)

M: the weight of the sample (g)

HPLC operating conditions:

Photodiode array detector: the quantitative wavelength 210 nm.

Column: COSMOSIL-HILIC, 5 µm, 4.6 mm i.d. × 25 cm.

Mobile phase: as section 2.4.

Flow rate: 1.0 mL/min.

Remark

1. Limits of quantitation (LOQs) for allantoin is 0.005%, and for urea is 0.25%.
2. Further validation should be performed when interference compounds appear in samples.