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Determination of prohibited aristolochic acid A in cosmetics - High performance liquid chromatography

化妆品中禁用物质马兜铃酸 A 的测定

高效液相色谱法

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Standardization Administration of the People's Republic of China.

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Determination of prohibited aristolochic acid A in cosmetics - High performance liquid chromatography

1 Scope

This Standard specifies reagents and materials, instruments and equipment, analysis procedures, calculation of result, recovery and precision, allowable difference, etc. for high performance liquid chromatography for prohibited aristolochic acid A in cosmetics.

This Standard is applicable to the determination of aristolochic acid A in skin care cosmetics such as creams, lotions, toning lotions.

The detection limit of this Standard method is 0.3mg/kg, and the limit of quantification is 1.0mg/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 of high performance liquid chromatography

The sample is ultrasonically extracted and filtered with methanol and detected by high performance liquid chromatograph equipped with a UV detector. Quantify by external standard method.

4 Reagents and materials

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

4.1 Methanol: chromatographically pure.

4.2 Glacial acetic acid.

6.3 Drawing of calibration curve

Respectively take 0.1mL, 0.5mL, 1.0mL, 2.0mL, 5.0mL of standard stock solutions (4.4). Use grade one water to set volume to 100mL. Prepare the standard working solutions of which the concentrations are 1.0μg/mL, 5.0μg/mL, 10.0μg/mL, 20.0μg/mL, 50.0μg/mL. Take standard working solutions at all levels into the high performance liquid chromatograph. Draw a calibration curve with peak area and solution concentration. For the liquid chromatogram of aristolochic acid A standard solution, see Figure B.1 in Annex B.

6.4 Sample determination

Take the sample solution (6.1) into the high performance liquid chromatograph. Determine the nature according to peak retention time and UV spectrum. Record chromatographic peak area. Obtain the corresponding concentration of aristolochic acid A from the calibration curve.

6.5 Qualitative confirmation

Conduct qualitative determination of sample by liquid chromatograph. When performing sample determination, if the detected peak value of the aristolochic acid A is consistent with the standard substance, and the sample chromatogram after deducting the background, the UV absorption spectrum of the material is consistent with the UV absorption spectrum of the reference material, it shall initially confirm the presence of aristolochic acid A. If necessary, positive samples shall be confirmed by liquid chromatography-mass spectrometry. See Annex C for confirmation test.

6.6 Blank test

Except for not taking sample, follow the above steps.

6.7 Parallel test

According to the above steps, perform the parallel test determination of the same sample.

7 Calculation of result

The content of aristolochic acid A in the sample is calculated by the chromatographic data processor or according to formula (1):

$$X=\frac{C\times V}{m} \qquad \cdots\cdots(1)$$

where,

Annex C

(informative)

Confirmation test

C.1 Liquid chromatography reference conditions

Liquid chromatography reference conditions are as follows:

a) Chromatographic column: EclipsePlus C18 (150mm x 2.1mm, 1.8μm), or equivalent column;

b) Mobile phase: 5mmol/L ammonium acetate solution (pH adjusted to 7.5 with ammonia water) - acetonitrile = 75-25 (volume ratio);

c) Flow rate: 0.2mL/min;

d) Column temperature: 30°C;

e) Injection volume: 5μL.

C.2 Mass spectrometry reference conditions

Mass spectrometry reference conditions are as follows:

a) Ion source: electrospray ion source (ESI);

b) Ionization mode: positive ion mode;

c) Drying gas temperature: 350°C;

d) Dry gas flow: 10L/min;

e) Sheath gas temperature: 300°C;

f) Sheath gas flow: 7L/min;

g) Capillary voltage: 4000V;

h) Data acquisition: multiple response monitoring (MRM) mode.

C.3 Qualitative determination

When the sample is tested, the sample solution is diluted appropriately. Perform determination of sample solution and standard working solution by liquid chromatography-mass spectrometry. If the selected ions are present and the

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