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Method of Test for Monacolin K in Foods

1. Scope

   This method is applicable to the determination of monacolin K (acid/lactone form, MKA/MKL) in monascus powder, capsules, and tablets.

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: Phenomenex luna C18 (2), 5 μm, 4.6 mm i.d. × 250 mm, or an equivalent product.

      2.1.2. Sonicator: frequency > 20 kHz, temperature control > 50℃.

      2.1.3. Centrifuge: rotary speed > 3000 rpm.

      2.1.4. Vortex mixer.

   2.2. Chemicals

      Acetone, HPLC grade;

      Methanol, HPLC grade;

      Phosphoric acid (85%), reagent grade;

      Sodium hydroxide, reagent grade;

      Deionized water, resistivity ≧ 18 MΩ·cm (at 25℃);

      Monacolin K lactone form (lovastatin or mevinolin), reference standard.

   2.3. Apparatus

      2.3.1. Volumetric flask: 10 mL, 25 mL, 100 mL and 1000 mL, amber.

      2.3.2. Centrifuge tube: 50 mL, PP.

      2.3.3. Sample vail: 2 mL, amber.

      2.3.4. Membrane filter: 0.45 μm, Nylon.

   2.4. Reagents

      2.4.1. 0.1 N sodium hydroxide

         Dissolve and dilute 4 g of sodium hydroxide with deionized water to 1000 mL.

      2.4.2. 0.1% phosphoric acid

Dilute 1.2 mL of phosphoric acid with deionized water to 1000 mL.

2.5. Mobile phase

Mix acetonitrile with 0.1% phosphoric acid at the ratio of 65:35 (v/v), and filter with a membrane filter.

2.6. Standard solution preparation

Transfer about 20 mg of monacolin K lactone form reference standard accurately weighed to a 10-mL volumetric flask. Dissolve and dilute to volume with acetonitrile as the standard stock solution. Store at -20℃ in the dark.

2.6.1. Monacolin K lactone form standard solution

Dilute appropriate volume of the standard stock solution with methanol to 1 ~ 200 μg/mL, as the monacolin K lactone form standard solution. Prepare prior to use.

2.6.2. Monacolin K acid form standard solution

Transfer 1 mL of the standard stock solution and 1 mL of 0.1 N sodium hydroxide in a 10-mL volumetric flask, vortex-mix, and ultrasonicate at 50℃ for 1 hr. Cool and dilute to volume with methanol. Then dilute to 1 ~ 100 μg/mL with methanol as the monacolin K acid form standard solutions. Prepare prior to use.

2.7. Sample solution preparation

Transfer about 0.2 g of the homogenized sample accurately weighed into a 25-mL volumetric flask. Add 25 mL of methanol, vortex-mix, and ultrasonicate for 30 min. Cool and dilute to volume with methanol. Centrifuge at 3000 rpm for 10 min. Collect the supernatant, and filter with a membrane filter as the sample solution.

2.8. Identification and quantification

Accurately inject 10 μL of the sample solution and the standard solutions into HPLC separately. Identify monacolin K based on the retention time and the absorption spectrum. Calculate the amount of monacolin K in the sample by the following formula:

$$\text{The amount of monacolin K in the sample (mg/g)} = \frac{(C_A + C_L) \times V}{M \times 1000}$$

Where,

C_A: the concentration of monacolin K acid form in the sample

solution calculated by the standard curve (μg/mL)

C_L: the concentration of monacolin K lactone form 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: UV 238 nm.

Column: Phenomenex luna C18 (2), 5 μm, 4.6 mm i.d. × 250 mm.

Mobile phase: as section 2.5.

Flow rate: 1.5 mL/min.

Remark

1. As monacolin K is unstable in methanol, the standard solutions and the sample solution should be analyzed within 24 hrs after preparation.
2. For samples with low content of monacolin K, it may increase the sample weight to analyze after performing the method validation.
3. Further validation should be performed when interference compounds appear in samples.