Trigonella foenum-graecum Seed 4-Hydroxyisoleucine Powdered Extract

Proposed For Comment Version 0.2

Trigonella foenum-graecum Seed 4-Hydroxyisoleucine Powdered Extract

DEFINITION
The article is a fraction of an extract of the dried ripe seeds of Trigonella foenum-graecum L. (Family Fabaceae). The extract is prepared using hydroalcoholic mixtures then further enriched in 4-hydroxyisoleucine using suitable means. It contains NLT 90.0% and NMT 110.0% of the labeled amount of 4-hydroxyisoleucine, calculated on the anhydrous basis.

POTENTIAL CONFOUNDING MATERIALS
None known

CONSTITUENTS OF INTEREST
Amino acids: 4-Hydroxyisoleucine, 4-hydroxyisoleucine lactone, arginine, histidine, and lysine

IDENTIFICATION
• Thin-Layer Chromatography—Amino Acids Profile
  Standard solution A: 0.5 mg/mL of USP 4-Hydroxyisoleucine RS in methanol
  Standard solution B: 50 mg/mL of USP Trigonella foenum-graecum Seed Powdered Extract RS in methanol. Sonicate for 10 min, centrifuge, and use the supernatant.
  Sample solution: 2.5 mg/mL of Trigonella foenum-graecum Seed 4-Hydroxyisoleucine Powdered Extract in methanol. Sonicate for 10 min, centrifuge, and use the supernatant.

  Chromatographic system
  (See Chromatography <621>, Thin-Layer Chromatography.)
  Adsorbent: Chromatographic silica gel mixture with an average particle size of 5 µm (HPTLC plates)
  Application volume: 2 µL each of Standard solution A and Standard solution B, and 4 µL of Sample solution, as 8-mm bands
  Relative humidity: Condition the plate to a relative humidity of about 33% using a suitable device.
  Temperature: 25°
  Developing solvent system: n-Butanol, acetic acid, and water (7:2:1)
  Developing distance: 6 cm
  Derivatization reagent: Ninhydrin reagent created by combining 0.3 g of ninhydrin, 95 mL of isopropanol, and 5 mL of glacial acetic acid.

  Analysis
  Samples: Standard solution A, Standard solution B, and Sample solution
  Apply the Samples as bands to a suitable HPTLC plate and dry in air. Develop the chromatograms in a saturated chamber, remove the plate from the chamber, and dry. Treat with Derivatization reagent,
heat for 2 min at 100°–105°, and examine under visible light and UV light at 366 nm.

**System suitability:** Under visible light, the chromatogram of the **Standard solution B** exhibits, in the lower half, five brown bands in the following order of increasing $R_f$: a minor band, the most intense band at an $R_f$ corresponding to the 4-hydroxyisoleucine band in the chromatogram of **Standard solution A**, and three less intense bands.

Under UV light at 366 nm, the chromatogram of **Standard solution B** exhibits, in the lower half, four bands in the following order of increasing $R_f$: a minor dark brown band, the most intense band as a dark brown band at an $R_f$ corresponding to the 4-hydroxyisoleucine band in the chromatogram of **Standard solution A**, and two less intense purple bands. The chromatogram of **Standard solution B** exhibits, in the upper half, three lemon-yellow bands, the one with the lowest $R_f$ appears diffuse and, in cases, may appear resolved into two bands.

**Acceptance criteria:** Under visible light, the chromatogram of the **Sample solution** exhibits, in the lower half, the most intense band as a brown band at an $R_f$ corresponding to the 4-hydroxyisoleucine band in the chromatogram of **Standard solution A**, and four additional brown bands corresponding to similar bands in the chromatogram of **Standard solution B**: a minor band at an $R_f$ below that of the 4-hydroxyisoleucine, and three less intense bands above the 4-hydroxyisoleucine band.

Under UV light at 366 nm, the chromatogram of the **Sample solution** exhibits, in the lower half, the most intense band as a dark brown band at an $R_f$ corresponding to the 4-hydroxyisoleucine band in the chromatogram of **Standard solution A**, and three additional bands corresponding to similar bands in the chromatogram of **Standard solution B**: a minor dark brown band at an $R_f$ below that of the 4-hydroxyisoleucine, and two less intense purple bands above the 4-hydroxyisoleucine band. The chromatogram of the **Sample solution** exhibits, in the upper half, three yellow bands corresponding to similar bands in the chromatogram of **Standard solution B**.

**ASSAY**

- **Content of 4-Hydroxyisoleucine**
  - **Solution A:** 0.1% Phosphoric acid in water (v/v)
  - **Solution B:** Acetonitrile
  - **Mobile phase:** See Table 1.

**Table 1**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Solution A (%)</th>
<th>Solution B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>20</td>
<td>40</td>
<td>60</td>
</tr>
</tbody>
</table>

**Reagent:** A mixture of acetonitrile, water, and triethylamine (40:12:8)

**Diluent:** Methanol and water (1:1)

**Standard solution:** Transfer about 2.0 mg of USP 4-Hydroxyisoleucine RS, accurately weighed, to a 100-mL volumetric flask, dissolve in 10 mL of **Reagent**, add 500 µL of phenyl isothiocyanate, and shake for 5 min. Add 60 mL of methanol, complete to volume with water, and mix.

**Sample stock solution:** Transfer about 60 mg of **Trigonella foenum-graecum** Seed 4-Hydroxyisoleucine Powdered Extract, accurately weighed, to a 10-mL volumetric flask, add 8 mL of **Diluent**, sonicate to dissolve, cool, complete to volume with **Diluent**, mix, and filter.

**Sample solution:** Transfer 2.0 mL of **Sample stock solution** to a 100-mL volumetric flask, add 10 mL of **Reagent** and 500 µL of phenyl isothiocyanate, and shake for 5 min. Add 60 mL of methanol, complete to
volume with water, and mix. Before Injection, pass through a membrane filter of 0.45-µm or finer pore size, discarding the first few mL of the filtrate.

**Chromatographic system**
(See Chromatography <621>, System Suitability.)

 Mode: LC
 Detector: UV 254 nm
 Column: 4.6-mm × 15-cm; 5-µm packing L1 (Similar to Zorbax Eclipse XD-C18, Luna C18(2) and Cosmosil C18-MS-II)
 Flow rate: 1.5 mL/min
 Injection volume: 20 µL

**System suitability**
Sample: Standard solution

**Suitability requirements**
Tailing factor: NMT 2.0 for the 4-hydroxyisoleucine peak, Standard solution
Relative standard deviation: NMT 2.0%, determined from the 4-hydroxyisoleucine peak in repeated injections, Standard solution

**Analysis**
Samples: Standard solution and Sample solution

Using the chromatogram of Standard solution, identify the retention time of the peak corresponding to 4-hydroxyisoleucine in the Sample solution chromatogram.

Calculate the percentage of 4-hydroxyisoleucine in the portion of *Trigonella foenum-graecum* Seed 4-Hydroxyisoleucine Powdered Extract taken:

\[
\text{Result} = \left( \frac{r_U}{r_S} \right) \times \left( \frac{C_S}{C_U} \right) \times d \times 100
\]

- \( r_U \) = peak area of 4-hydroxyisoleucine from the Sample solution
- \( r_S \) = peak area of 4-hydroxyisoleucine from Standard solution
- \( C_S \) = concentration of 4-hydroxyisoleucine in Standard solution (mg/mL)
- \( C_U \) = concentration of *Trigonella foenum-graecum* Seed 4-Hydroxyisoleucine Powdered Extract in the Sample stock solution (mg/mL)
- \( d \) = dilution factor to prepare the Sample solution from the Sample stock solution

Calculate the percentage of the labeled amount of 4-hydroxyisoleucine in the Extract:

\[
\text{Result} = (P/L) \times 100
\]

- \( P \) = content of 4-hydroxyisoleucine as determined above (%)
- \( L \) = labeled amount of 4-hydroxyisoleucine (%)

**Acceptance criteria:** 90.0%–110.0% of the labeled amount of 4-hydroxyisoleucine, on the anhydrous basis

**CONTAMINANTS**
- **Elemental Impurities—Procedures <233>**

Acceptance criteria
Arsenic: NMT 2.0 µg/g
**Cadmium:** NMT 1.0 µg/g  
**Lead:** NMT 10.0 µg/g  
**Mercury:** NMT 1.0 µg/g

- **Articles of Botanical Origin,** *General Method for Pesticide Residues Analysis* <561>: Meets the requirements
- **Microbial Enumeration Tests** <61>: The total aerobic bacterial count does not exceed 104 cfu/g and the total combined molds and yeasts count does not exceed 102 cfu/g.
- **Tests for Specified Microorganisms** <62>: Meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli*

**Specific Tests**
- **Water Determination,** *Method Ia* <921>: NMT 6.0%

**Additional Requirements**
- **Packaging and Storage:** Preserve in well-closed containers, protected from light and moisture, and store at room temperature.
- **Labeling:** The label states the Latin binomial and, following the official name, the part of the plant from which the article was derived. It meets other labeling requirements under *Botanical Extract* <565>.
- **USP Reference Standards** <11>
  - USP 4-Hydroxyisoleucine RS
  - USP *Trigonella foenum-graecum* Seed Powdered Extract RS

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