Trigonella foenum-graecum Seed Powdered Extract

Proposed For Comment Version 0.2

Trigonella foenum-graecum Seed Powdered Extract

DEFINITION
The article is prepared from the dried ripe seeds of *Trigonella foenum-graecum* L. (Family Fabaceae) by extraction with hydroalcoholic mixtures. The ratio of starting crude plant material to Powdered Extract is between 5:1 and 4:1. It contains NLT 90.0% and NMT 110.0% of the labeled amount of 4-hydroxyisoleucine, calculated on the anhydrous basis. It may contain suitable added carriers.

POTENTIAL CONFOUNDING MATERIALS
None known

CONSTITUENTS OF INTEREST
Amino acids: 4-Hydroxyisoleucine, 4-hydroxyisoleucine lactone, arginine, histidine, and lysine
Steroidal saponins: Trigoneneoside IIa, Ib; graecunins H, I, J, K, L, M, N; trigofenosides A, D, F, G; protogracillin; protodioscin; and diosgenin
Alkaloids: Trigonelline, gentianine, and carpaine
Flavonoids: Apigenin, luteolin, orientin, quercetin, vitexin, and isovitexin
Carbohydrates: Mainly mucilage

IDENTIFICATION

A. 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**: 50 mg/mL of *Trigonella foenum-graecum* Seed Powdered Extract in methanol. Sonicate for 10 min, centrifuge, and use the supernatant.

[NOTE—Save the Sample solution for use in Identification B and the Specific Tests—Presence of Trigonelline.]

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, 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 - 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 Standard solution B exhibits, in the lower half, five brown bands in the following order of increasing Rf: a minor band, the most intense band at an Rf 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 the Standard solution B exhibits, in the lower half, four bands in the following order of increasing Rf: a minor dark brown band, the most intense band as a dark brown band at an Rf 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 Rf, 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 Sample solution exhibits, in the upper half, three yellow bands corresponding to similar bands in the chromatogram of Standard solution B.

B. Thin-Layer Chromatography—Steroidal Saponins Profile

Standard solution A: 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: Use the Sample solution prepared in Identification A.

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, 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: Dichloromethane, methanol, and water (18:8:1)

Developing distance: 6 cm

Derivatization reagent: Anisaldehyde reagent created by combining 85 mL of ice-cooled methanol mixed with 10 mL of glacial acetic acid, 5 mL of sulfuric acid, and 0.5 mL of $p$-anisaldehyde.

Analysis

Samples: Standard solution A 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 3 min at 100°, and examine under visible light and UV light at 366 nm.

System suitability: Under visible light, the chromatogram of Standard solution A exhibits, in the lower half, four intense bands in the following order of increasing $R_F$: a brown band, a brown band due to protogracillin, a brown band due to protodioscin, and a violet band. The chromatogram of Standard solution A exhibits, in the upper half, two violet bands and a third violet band close to the solvent front.

Under UV light at 366 nm, the chromatogram of Standard solution B exhibits, in the lower half, three blue fluorescent bands interspersed with three brown bands due to saponins, a pink band, and a broad blue fluorescent band.

Acceptance criteria: Under visible light, the chromatogram of the Sample solution exhibits three intense brown bands and a violet band in the lower half, and three violet bands in the upper half. All of these bands correspond to similar bands in the chromatogram of Standard solution A.

Under UV light at 366 nm, the chromatogram of the Sample solution exhibits, in the lower half, three blue fluorescent bands interspersed with three brown bands, a pink band, and a broad blue fluorescent band. All of these bands correspond to similar bands in the chromatogram of Standard solution A.

ASSAY

C. 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 0.2 g of *Trigonella foenum-graecum* Seed 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 5.0 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 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 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:

\[
\text{Result} = \left( \frac{P}{L} \right) \times 100
\]

- \(P\) = 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 10^4 cfu/g and the total combined molds and yeasts count does not exceed 10^2 cfu/g.
- **TESTS FOR SPECIFIED MICROORGANISMS <62>:** Meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli*

**SPECIFIC TESTS**
- **PRESENCE OF TRIGONELLINE**
- **Standard solution A:** 1.5 mg/mL of USP Trigonelline Hydrochloride 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:** Use the *Sample solution* prepared in *Identification A*.

**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:** 5 µL, 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:** A mixture of isopropyl alcohol, methanol, and water (4:1:4)
- **Developing distance:** 6 cm
- **Derivatization reagent:** Dragendorff’s reagent—suspend 1.7 g of bismuth oxynitrate and 20 g of (+)-tartaric acid in 40 mL of water. To the suspension add 40 mL of a 40% solution of potassium iodide in water (w/v), stir for 1 h and filter. This stock solution may be kept for several days protected from light. Immediately before use, mix 5 mL of the stock solution with 15 mL of water.

**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, dry, and examine under UV light at 254 nm. Derivatize with *Derivatization reagent,* dry, and examine under white light.

- **System suitability:** Under UV light before derivatization, the chromatogram of *Standard solution B* exhibits, in the lower half, a quenching band at an *R*$_f$ similar to the trigonelline band in the chromatogram of *Standard solution A*.

  Under white light after derivatization, the chromatogram of *Standard solution B* exhibits, in the lower half, an orange-red band corresponding in *R*$_f$ and color to the trigonelline band in the chromatogram of *Standard solution A*.

**Acceptance criteria:** Under UV light before derivatization, the chromatogram of the *Sample solution* exhibits a quenching band corresponding in *R*$_f$ to the trigonelline band in the chromatogram of *Standard solution A*.

Under white light after derivatization, the chromatogram of the *Sample solution* exhibits an orange-red band corresponding in *R*$_f$ and color to the trigonelline band in the chromatogram of *Standard solution A*.

• **WATER DETERMINATION, Method la** <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
  - USP Trigonelline Hydrochloride RS

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