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Fenugreek Seed Powder

DEFINITION

Fenugreek Seed Powder consists of the dried ripe seeds of *Trigonella foenum-graecum* L. (Fam. Fabaceae), reduced to powder or very fine powder. It contains NLT 0.2% of 4-hydroxyisoleucine, calculated on the dried basis.

IDENTIFICATION

• A. [HPTLC FOR ARTICLES OF BOTANICAL ORIGIN \(203\)](#).

Standard solution A: 0.5 mg/mL of [USP 4-Hydroxyisoleucine RS](#) in an ethanol and water (7:3) mixture

Standard solution B: 50 mg/mL of [USP Trigonella Foenum-graecum Seed Dry Extract RS](#) in an ethanol and water (7:3) mixture. Sonicate for 10 min, centrifuge, and use the supernatant.

Sample solution: Suspend about 1 g of Powder in 5 mL of an ethanol and water (7:3) mixture, and incubate at 50° for 15 min. Centrifuge, and use the supernatant.

[NOTE—*Standard solution B* and the *Sample solution* may also be used for *Identification test B* and for *Specific Tests, Presence of Trigonelline*.]

Chromatographic system

Adsorbent: Chromatographic silica gel with an average particle size of 5 µm (HPTLC plate)¹

Application volume: 2 µL each of *Standard solution A* and *Standard solution B*, and 4 µL of the *Sample solution*, as 8-mm bands

Relative humidity: Condition the plate to a relative humidity of 33% using a suitable device.

Temperature: Ambient, not to exceed 30°

Developing solvent system: A mixture of *n*-butanol, acetic acid, and water (7:2:1)

Derivatization reagent: A solution of 0.3% ninhydrin in a mixture of isopropanol and glacial acetic acid (19:1)

System suitability

Samples: *Standard solution A* and *Standard solution B*

Suitability requirements: Under white light, the derivatized chromatogram of *Standard solution B* displays, in its lower half, five or six brown bands; the darkest band corresponding to the 4-hydroxyisoleucine band in the chromatogram of *Standard solution A*. Under long-wave UV (365 nm), the derivatized chromatogram of *Standard solution B* exhibits, in its lower half, four or five brown bands; the darkest band corresponding to the 4-hydroxyisoleucine band in the chromatogram of *Standard solution A*. In the upper half of the chromatogram, three diffuse yellow to orange-yellow bands are seen, the middle one being the most intense.

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Apply the *Samples* as bands, and dry in air. Condition at relative humidity at about 33%. Develop in a saturated chamber, until the solvent front has migrated over a path of 6 cm. Air-dry, treat with *Derivatization reagent*, heat for 3 min at 105°, and immediately examine under white light and under long-wave UV light (365 nm).

Acceptance criteria: Under white light, the derivatized chromatogram of the *Sample solution* appears monochromatic, with the bands differing in intensity, but not the color, which is uniformly reddish-brown. In the lower third of the plate, two or three thin bands are seen, proximate to the origin, followed by a very intense band due to 4-hydroxyisoleucine, coincident with the corresponding bands in *Standard solution A* and *Standard solution B*, and comparable in intensity to the band in the *Standard solution B* chromatogram. Two lightly colored bands, one just above the 4-hydroxyisoleucine band, another further upwards, are seen. The upper half of the plate is devoid of discernible features. Under long-wave UV (365 nm), the derivatized chromatogram of the *Sample solution* exhibits, in its lower half, two or three light reddish-brown bands followed by the darker-brown intense band due to 4-hydroxyisoleucine, coincident with the corresponding bands in *Standard solution A* and *Standard solution B*. Above it, two lighter brown and somewhat diffuse bands appear. In the upper third of the plate, three yellowish-orange bands are seen, corresponding in position and color to those observed in *Standard solution B*.

• B. [HPTLC FOR ARTICLES OF BOTANICAL ORIGIN \(203\)](#).

Standard solution: 50 mg/mL of [USP Trigonella Foenum-graecum Seed Dry Extract RS](#) in an ethanol and water (7:3) mixture. Sonicate for 10 min, centrifuge, and use the supernatant.

Sample solution: Suspend about 1 g of Powder in 5 mL of an ethanol and water (7:3) mixture, and incubate at 50° for 15 min. Centrifuge, and use the supernatant.

[NOTE—The *Standard solution* and *Sample solution* may also be used for *Identification test A* and for *Specific Tests, Presence of Trigonelline*.]

Chromatographic system

Adsorbent: Chromatographic silica gel with an average particle size of 5 µm (HPTLC plate)

Application volume: 2 µL each of the *Standard solution* and the *Sample solution*, as 8-mm bands

Relative humidity: Condition the plate to a relative humidity of 33% using a suitable device.

Temperature: Ambient, not to exceed 30°

Developing solvent system: A mixture of dichloromethane, methanol, and water (18:8:1)

Derivatization reagent: A mixture of methanol, glacial acetic acid, sulfuric acid, and *p*-anisaldehyde (170:20:10:1). Prepare on an ice bath, and mix well.

System suitability

Sample: *Standard solution*

Suitability requirements: Under white light, the derivatized chromatogram of the *Standard solution* exhibits, in its lower half, three or four lightly shaded bands. Under long-wave UV (365 nm), the derivatized chromatogram of the *Standard solution* exhibits, in its lower third, two diffuse bands of blue fluorescence, and another blue fluorescent band in the middle of the plate.

Analysis

Samples: *Standard solution* and *Sample solution*

Apply the *Samples* as bands, and dry in air. Condition at relative humidity at about 33%. Develop in a saturated chamber, until the solvent front has migrated over a path of 6 cm. Air-dry, treat with *Derivatization reagent*, heat for 3 min at 105°, and immediately examine under white light and under the long-wave UV light (365 nm).

Acceptance criteria: Under white light, the chromatogram of the *Sample solution*, in its lower third, shows two medium-intensity bands immediately next to the application line. Further upwards, two closely spaced, less intense, bands are followed by a more prominent band, and another pair of closely spaced lighter bands, which may merge. In the middle third of the chromatogram, a medium-intensity band is followed by a faint diffuse band. In the upper third of the chromatogram, two well-defined bands of medium intensity are observed. Under UV light (365 nm), the lower half of the chromatogram features three or four deep-blue fluorescent bands of varying intensity interspersed with greyish-brown zones.

COMPOSITION

• CONTENT OF 4-HYDROXYISOLEUCINE

Solution A: 0.1% Phosphoric acid in water

Solution B: Acetonitrile

Mobile phase: See [Table 1](#).

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80.0	20.0
20	40.0	60.0
21	80.0	20.0
25	80.0	20.0

Diluent: Methanol and water (1:1)

Reagent: A mixture of acetonitrile, water, and triethylamine (10:3:2)

Standard solution: Transfer about 4.0 mg of [USP 4-Hydroxyisoleucine RS](#), accurately weighed, into a 50-mL volumetric flask, and dissolve in 5 mL of the *Diluent*. Add 10 mL of *Reagent* and 0.5 mL of phenyl isothiocyanate, and shake for 5 min. Add 30 mL of methanol, adjust with water to volume, and mix well.

Sample stock solution: Transfer about 2.0 g of accurately weighed Powder into a centrifuge tube. Add 8 mL of *Diluent*, place on a water bath at 65° for 5 min, sonicate for 5 min, and centrifuge. Retain the supernatant, and repeat extraction with 8 mL of *Diluent* two more times. Combine all three extracts in the 25-mL volumetric flask, dilute with *Diluent* to volume, and mix well.

Sample solution: Transfer 5.0 mL of *Sample stock solution* into a 50-mL volumetric flask, add 10 mL of *Reagent* and 0.5 mL of phenyl isothiocyanate, and shake for 5 min. Add 30 mL of methanol, dilute with water to volume, and mix well. Pass through a nylon filter having a 0.45-µm or finer pore size, discarding the initial few mL of the filtrate.

Chromatographic system

(See [Chromatography \(621\)](#), [System Suitability](#).)

Mode: HPLC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: Ambient

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*