USP botanical quality standards:
contributions in quality control & safe use of botanicals

Hellen Oketch-Rabah, PhD
Senior Scientific Liaison, Dietary Supplements, Herbal Medicines
UNITED STATES PHARMACOPEIA

2017 NCAC-SOT Spring Symposium
April 19, 2017 Lister Hill Auditorium NIH Campus, Bethesda, MD
MISSION

To improve global health through public standards and related programs that help ensure the quality, safety, and benefit of medicines and foods.
**DISCLAIMER:**

Because USP text and publications may have legal implications in the U.S. and elsewhere, their language must stand on its own. The USP shall not provide an official ex post facto interpretation to one party, thereby placing other parties without that interpretation at a possible disadvantage. The requirements shall be uniformly and equally available to all parties.

In addition, USP shall not provide an official opinion as to whether a particular article does or does not comply with compendial requirements, except as part of an established USP verification or other conformity assessment program that is conducted separately from and independent of USP's standard-setting activities.

Certain commercial equipment, instruments or materials may be identified in this presentation to specify adequately the experimental procedure or illustrate a point. Such identification does not imply approval, endorsement, or certification by USP of a particular brand or product, nor does it imply that the equipment, instrument or material is necessarily the best available for the purpose or that any other brand or product was judged to be unsatisfactory or inadequate.

Any views expressed in this presentation are solely the authors’ views.
I wish to thank the following for their contributions:

*USP:*

Dr. Gabriel I. Giancaspro
Dr. Nandu D. Sarma
Mr. Doug Podolsky

*Students*

Ms Mary Pothen, MS (Pharm. D candidate, University of Maryland)
Ms. Kimberly Uwe, MS (Pharm. D candidate, University of Maryland)
What is a USP DS quality standard: monographs, general notices and general chapters, Ref. materials

Development of USP DS quality monograph preceded by USP Admission Evaluation that is based on safety evaluation

How USP monograph contributes to public safety

Message: USP DS quality standards for public safety
What is a standard?

A standard is a document that provides requirements, specifications, guidelines or characteristics that can be used consistently to ensure that materials, products, processes and services are fit for their purpose.

The International Organization for Standardization
www.iso.org/iso/home/standards.htm

USP Public standards include Monographs that provide specifications:

- Specifications
  - Tests
  - Analytical Procedures
  - Acceptance Criteria
USP Standards include:

Monographs
Related USP General Chapters & General Notices
Reference materials

To *comply* with USP quality standard an article must conform to all requirements: tests in a monograph (incl. referenced chapters) and General Notices
Dietary Supplements Monograph Development

CONSTANT COMMUNICATION

STAKEHOLDERS
- ACADEMIA
- INDUSTRY
- REGULATORY AUTHORITIES

USP Nomenclature

MONOGRAPH CANDIDATE*
(FOOD OR DIETARY SUPPLEMENT INGREDIENT)

COLLECTION OF DATA

MONOGRAPH DRAFT AND INTERNAL USP REVIEW

MONOGRAPH PROPOSED
FOOD CHEMICAL CODEX (FCC) OR USP-NF FORUM FOR PUBLIC COMMENTS

PF
FCCF

MONOGRAPH APPROVED
BY EXPERT COMMITTEES AND PUBLISHED IN THE FCC OR USP-NF

REQUEST FOR REVISION
A REVIEW REQUEST CAN BE INITIATED BY EITHER USP OR STAKEHOLDERS FOR ANY MONOGRAPH PUBLISHED IN FCC OR USP-NF.

USP

Admission Evaluation

Expert Panel Recommendation


*Monograph candidates have to meet USP's criteria for inclusion in either FCC or USP-NF (criteria include approved legal status, no known safety concerns, commercially available ingredient, clear identification and description, among others).
Information (evidence based):

1. *Ingredient characterization*: characteristics of the material under review (botanical power, extract etc.)
2. *Exposure*: intake levels, route of administration (relevant is by mouth).
3. *Human data*: safety studies, clinical studies, post-marketing surveillance, adverse events (case reports, AER portals), interactions etc.
4. *Pharmacological data*: reproductive toxicity, experimental animal studies, pharmacokinetics, safety index, and presence of toxic constituents
5. *Contemporaneous extent of use*: globally and in the U.S.; including misuse and abuse
6. *Historical use*: Globally
7. *Regulatory status*: in the U.S. and other countries: regulatory actions, OTC status, GRAS status, etc.
8. *Presence in other pharmacopeia*
USP Quality Attributes in Specifications in a USP Monograph

- **USP Compendial Standards: Monographs Contains Specifications**
  - Identity (Identification)
  - Assay for content (Purity)
  - Composition ( Constituents)
  - Absence of Contaminants ( Contaminants)
  - Impurities ( impurities)
  - Specific Tests
  - Other requirements (labeling and packaging)

- Consistent with GMPs for DS
<table>
<thead>
<tr>
<th>USP Term(s)</th>
<th>USP Monograph Test Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Identification</strong></td>
<td>The monograph section titled “Identification” may consist of one or more (orthogonal) tests to verify the identity of articles. Failure of the article to meet one or more of the tests under the identification section indicates that the article is mislabeled and/or adulterated.</td>
</tr>
<tr>
<td><strong>Assay or Content of …</strong></td>
<td>The USP monograph tests for Assay and Content of (specific constituent or marker) are used to measure the amount of a substance in an ingredient, which in FDA’s language is the overall purity of the ingredient. Conversely, the USP monograph tests for impurities provide means to determine the portion of an ingredient that is not the intended component.</td>
</tr>
<tr>
<td><strong>Composition</strong></td>
<td>Composition applies to multiple constituents in an ingredient.</td>
</tr>
<tr>
<td><strong>Contaminants</strong></td>
<td>Contaminants may arise from any source extraneous to the manufacturing process and that is introduced by contamination or adulteration. Contaminants can be classified as microbiological (e.g., objectionable microorganisms) or chemical (e.g., pesticides). Analytical procedures for the determination of contaminants can be quantitative assays or limit tests.</td>
</tr>
<tr>
<td><strong>Impurities</strong></td>
<td>Impurities may arise from the manufacture (e.g., reagents, byproducts) or storage (e.g., degradation) of an article. Impurities can be classified as organic or inorganic. Setting impurity or degradation product limits for articles is based on chemistry and safety concerns. Analytical procedures for the determination of impurities can be quantitative assays or limit tests.</td>
</tr>
<tr>
<td><strong>Specific Tests</strong></td>
<td>Specific tests are sometimes included to further characterize an article, such as tests for the chemo-physical nature of fats and fixed oils (e.g., specific gravity, melting temperature), and the rancidity of fats and fixed oils (e.g., Peroxide Value, Anisidine Value).</td>
</tr>
</tbody>
</table>
# Quality attributes in USP monographs for dietary supplements

<table>
<thead>
<tr>
<th>USP Term(s)</th>
<th>USP Monograph Test Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identification</td>
<td>See <em>Identification</em> in the previous slide</td>
</tr>
<tr>
<td>Strength, Assay</td>
<td>Assay test procedures are used to measure the strength of a dietary ingredient in a dietary supplement. Assay test procedures measure the concentration or amount of a dietary ingredient per unit serving of a dietary supplement. Strength applies only to finished dosage forms (dietary supplements).</td>
</tr>
<tr>
<td>Contaminants</td>
<td>See <em>Contaminants</em> in the previous slide</td>
</tr>
<tr>
<td>Impurities</td>
<td>See <em>Impurities</em> in the previous slide</td>
</tr>
<tr>
<td>Specific Tests</td>
<td>See <em>Specific Tests</em> in the previous slide</td>
</tr>
<tr>
<td>Performance</td>
<td>Performance tests (e.g., disintegration, dissolution) are quality control tests to assess the release characteristics of finished products.</td>
</tr>
</tbody>
</table>
**Example of a USP Botanical Monograph**

e.g. FENUGREEK

<table>
<thead>
<tr>
<th>USP Term(s)</th>
<th>Test Characteristics in Probiotic Monographs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identification</td>
<td>Identification (e.g. Genus species and Family), TLC/HPTLC, HPLC, macroscopy and microscopy</td>
</tr>
<tr>
<td>Assay or Content of …</td>
<td>Chromatography-HPLC, GC, etc.</td>
</tr>
<tr>
<td>Composition</td>
<td>Chromatography-HPLC, GC, etc.</td>
</tr>
<tr>
<td>Contaminants</td>
<td>Microbial contaminants (e.g. <em>E. coli</em>, <em>Salmonella</em> species)</td>
</tr>
<tr>
<td>Impurities</td>
<td>Heavy metals, herbicides and pesticides</td>
</tr>
<tr>
<td>Specific Tests</td>
<td>Macroscopy and microscopy, loss on drying, ash, etc.</td>
</tr>
</tbody>
</table>
USP General Chapters

- Specific for Dietary Supplements

- Microbial Enumeration Tests, Nutritional and Dietary Supplements

- Microbiological Procedures for Absence of Specified Microorganisms, Nutritional and Dietary Supplements

- Microbiological Attributes of Non-sterile Nutritional and Dietary Supplements

- Disintegration and Dissolution of Dietary Supplements

- Weight Variation of Dietary Supplements

- Elemental Impurities in Dietary Supplements

- Manufacturing Practices for Dietary Supplements
USP General Chapters

- Non Specific for Dietary Supplements

- HPTLC for Botanical Identification

- Elemental Impurities - Procedures

- Arsenic

- Lead

- Folic acid

- Residual Solvents

- Articles of Botanical Origin

- Identification of Articles of Botanical Origin

- Chromatography

- Loss on Drying
Add the following:

**Fenugreek Seed**

**DEFINITION**
Fenugreek Seed consists of the dried ripe seeds of *Trigonella foenum-graecum* L. (Fam. Fabaceae). It contains NLT 0.2% of 4-hydroxyisoleucine, calculated on the dried basis.

**IDENTIFICATION**

- **A. HPTLC for Articles of Botanical Origin** (203) - **Amino Acid Profile**
  
  Standard solution A: 0.5 mg/mL of USP 4-Hydroxyisoleucine RS in an ethanol and water (7:3) mixture. Sonicate for 10 min. Cool to 30°C. Centrifuge, and use the supernatant.

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

  The Standard solution B and the Sample solution may also be used for Identification test B and for Specific Tests, Presence of Trigonelline.

**Chromatographic system**

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

**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.

**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 dark 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 environment until the solvent front has moved about 30 cm along the plate. Dust with Derivatization reagent, heat for 3 min at 105°C, 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 upper third of the plate, two or three thin bands are seen, proximate to the origin, followed by a diffuse, more 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 or three 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 darkest 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, the yellowish-orange

1 Suitable commercially available plates are HPTLC Silica Gel 60 F254, from EM Millipore (e.g., Part No. 1.05642.0001).
bands are seen, corresponding in position and color to those observed in Standard solution B.

**B. **

**BOTANICAL PROFILE**

**Saponins Profile**

**Standard solution:** 50 mg/mL of USP Trigonella Foenum-graecum Seed Powdered Extract RS in an ethan-

ol-water (7:3) mixture. Sonicate for 10 min, centrifuge, and use the supernatant.

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

[Note: Only a 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, 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 dichloro-
me-thane, methanol, and water (18:8:1)

**Derivatization reagent:** A mixture of methanol, gla-

cic acid, sulfuric acid, and p-anisaldehyde (1:20:10:1). 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:** 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 approximately 6 cm. Air-dry, treat with the 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 chromatogram of the Sample solution, in its lower third, shows two medium-intensity bands immediately next to the application line. Further upwards, two more closely spaced bands are followed by a more prominent band, and another pair of closely spaced lighter bands, which may be nearly in the middle third. 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>
<thead>
<tr>
<th>Time (min)</th>
<th>Solution A (%)</th>
<th>Solution B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>80.0</td>
<td>20.0</td>
</tr>
<tr>
<td>20</td>
<td>20.0</td>
<td>40.0</td>
</tr>
</tbody>
</table>

**Table 1 (Continued)**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Solution A (%)</th>
<th>Solution B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>80.0</td>
<td>20.0</td>
</tr>
<tr>
<td>25</td>
<td>80.0</td>
<td>20.0</td>
</tr>
</tbody>
</table>

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

**Reagent:** A mixture of acetonitrile, water, and triethyl-

amine (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 Dilu-

tent. Add 10 mL of Reagent and 0.5 mL of phenyl isothio-

cyanate, 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 Fenu-

greek Seed, finely powdered and accurately weighed, 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 solu-

tion into a 50-mL volumetric flask, add 10 mL of Rea-

gent 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

**Relative standard deviation:** NMT 2.0% determined for the 4-hydroxyisoleucine peak in replicate injec-

tions, Standard solution

**Calculate the percentage of 4-hydroxyisoleucine in the portion of Fenugreek Seed taken:**

\[ \text{Result} = \left( \frac{ru}{rs} \right) \times \left( \frac{V}{W} \right) \times D \times 100 \]

\[ ru = \text{peak area of 4-hydroxyisoleucine from the Sample solution} \]

\[ rs = \text{peak area of 4-hydroxyisoleucine from the Standard solution} \]

\[ C_s = \text{concentration of USP 4-Hydroxyisoleucine RS in the Standard solution (mg/mL)} \]

\[ V = \text{volume of the Sample stock solution (mL)} \]

\[ W = \text{weight of Fenugreek Seed taken to prepare the Sample stock solution (mg)} \]

\[ D = \text{dilution factor to prepare the Sample solution from the Sample stock solution, 10} \]
Acceptance criteria: NLT 0.2% on the dried basis

**CONTAMINANTS**

- **Elemental Impurities—Procedures (233)**
  - 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, Pesticide Residue Analysis (561):** Meets the requirements.

**Microbiological Tests (2021):** The total aerobic bacterial count does not exceed 10^5 cfu/g, the total combined molds and yeasts count does not exceed 10^3 cfu/g, and the bile tolerant Gram-negative bacterial count does not exceed 10^3 cfu/g.

**Absence of Specified Microorganisms (2022):** Meets the requirements of the tests for absence of Salmonella species and Escherichia coli.

**SPECIFIC TESTS**

- **HPTLC for Articles of Botanical Origin (203)—Prescence of Trigonelline**
  - Standard solution A: 5 mg/mL of USP Trigonelline RS in 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
  - Sample solution: Suspend about 1 g of Fenugreek Seed, finely powdered, in 5 mL of an ethanol and water (7:3) mixture. Sonicate for 10 min, centrifuge, and use the supernatant.

  **NOTE:** Standard solution B and the Sample solution may also be used for Identification test A and for Identification test B.

**Chromatographic system**

- **Adsorbent:** Chromatographic silica gel with an average particle size of 5 μm (HPTLC plate)
- **Application volume:** 5 μL, as 8-mm bands
- **Relative humidity:** Condition the plate to a relative humidity not exceeding 33% using suitable desiccant
- **Temperature:** Ambient, not to exceed 30°C

**Developing solvent:** A mixture of isopropyl alcohol, methanol, and water (4:1:4)

**System suitability**

- **Samples:** Standard solution A and Standard solution B
- **Suitability requirements:** Under short-wave UV light, the chromatogram of the Standard solution A displays a quinoid compound corresponding to the trigonelline band in the chromatograms of Standard solution A and Standard solution B.

**Analysis**

- **Samples:** Standard solution A, Standard solution B, and Sample solution
- **Procedure:** 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, and examine under short-wave UV light (254 nm).

**Acceptance criteria:** Under short-wave UV light, the chromatogram of the Sample solution displays a quinoid compound corresponding to the trigonelline band in the chromatograms of Standard solution A and Standard solution B.

**BOTANICAL CHARACTERISTICS**

**Macro:** The seeds are oblong, 3–5 mm long, 2–3 mm wide, 1.5–2.0 mm thick, with rounded corners, smooth, dull yellowish-brown to reddish-brown. They are flattened and have a very characteristic rhomboidal outline. Nearly in the center of one of the long, narrow sides is a small depression in which both hilum and micropyle are situated, the former being distinctly visible. The edge of the cut surface is continued in the form of a furrow running diagonally across part of each of the adjoining sides, dividing the seed into two unequal lobes. A cut made transversely to pass through both lobes reveals two accumbent cotyledons in the larger lobe, and the radicle in the smaller lobe. Both are yellowish in color, with an outer brown layer, and surrounded by a translucent endosperm, which also separates the radicle from the cotyledons.

**Microscopic:** Transverse section shows an epidermis of palisade cells, one layer thick, with thick cuticle and thick lamellated walls, and a relatively large lumen at the lower part. Longitudinal pit-canals fine and close. Seed coat: inner layer of basket-like cells, with bar-like thickening on the radial walls, followed by a parenchymatous layer. Endosperm consists of a layer of thick-walled cells containing aleurone grains, several layers of polyhedral cells with stratified mucilaginous contents and thickened walls. Cotyledons of parenchymatous cells containing fixed oil globules, and aleurone grains up to 10 μm in diameter. The testa is composed of several layers of thin-walled cells which appear similar in sectional view but in surface view the layers show structural differences: some are composed of rectangular cells with slightly thickened and beaded walls; other layers include thin-walled polygonal cells which may be very irregular in size or may form irregular intercellular spaces.

**ARTICLES OF BOTANICAL ORIGIN, Foreign Organic Matter (561):** NMT 2.0%

**LOSS ON DRYING (731):**

- **Sample:** 1 g
- **Analysis:** Dry the Sample at 105°C for 2 h.
- **Acceptance criteria:** NMT 12.0%

**ARTICLES OF BOTANICAL ORIGIN, Total Ash (561):** NMT 5.0%

**ARTICLES OF BOTANICAL ORIGIN, Alcohol-Soluble Extractives (Method 1 (561):** NMT 5.0%

**ARTICLES OF BOTANICAL ORIGIN, Water-Soluble Extractives (Method 1 (561): NLT 9.0%

**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 drug is derived.
- **USP Reference Standards (11):**
  - USP 4-Hydroxyisoleucine RS
  - USP Trigonella Foenum-graecum Seed Dry Extract RS
  - USP Trigonelline RS

**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

  - **NOTE:** Precipitate for 10 min, centrifuge, and use the supernatant.

  **Add the following:**
Fig. 1 Dried ripe seeds of *Trigonella foenum-graecum* L.
A. Seeds  B. Magnified seeds  C. Magnified fracture of seeds  D. Magnified cotyledons and radicle

Fig. 2 Microscopic features of transverse section of *Trigonella foenum-graecum* seed
A. Sketch  B. Illustration of transverse section  C. Magnification showing epidermis of testa and subepidermal layer
Fig. 3 Microscopic features of powder of *Trigonella foenum-graecum* seed

a. Features under a light microscope  b. Features under a polarized light microscope

1-1. Palisade cells (lateral view)  1-2. Palisade cells (surface view)  1-3. Palisade cells (bottom view)  2. Hypodermis  2-1.

Basket-like cells (lateral view)  2-2. Basket-like cells (bottom view)  3. Parenchymatous cells  4. Endosperm cells
Presence of Trigonelline

![Typical HPTLC chromatograms](image)

**Fig. 7 Typical HPTLC chromatograms**

**Track assignment:** 1) USP Trigonelline RS, 1.5 mg/mL; 2) USP *Trigonella foenum-graecum* Seed Dry Extract RS; 3–6) Fenugreek seed (commercial samples)

Non-USP Method - Flavonoid Profile

![Typical HPTLC chromatograms](image)

**Fig. 8 Typical HPTLC chromatograms**

a. Image of underivatized plate under 254 nm  b. Image of derivatized plate under UV 365 nm

**Track assignment:** 1) USP Vitexin RS, 0.5 mg/mL; 2) USP *Trigonella foenum-graecum* Seed Dry Extract RS, 50 mg/mL; 3–6) Fenugreek seed (commercial samples)
Fig. 5  Typical HPTLC chromatograms

**Track assignment:** 1) USP 4-Hydroxyisoleucine RS; 2) USP *Trigonella foenum-graecum* Seed Dry Extract RS; 3–6) Fenugreek seed (commercial samples)

Fig. 6  Typical HPTLC chromatograms

**Track assignment:** 1) USP *Trigonella foenum-graecum* Seed Dry Extract RS; 2–5) Fenugreek seed (commercial samples)
<table>
<thead>
<tr>
<th>USP Term(s)</th>
<th>USP Monograph Test Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identification</td>
<td>The monograph section titled “Identification” may consist of one or more (orthogonal) tests to verify the identity of articles. Failure of the article to meet one or more of the tests under the identification section indicates that the article is mislabeled and/or adulterated.</td>
</tr>
<tr>
<td>Assay or Content of …</td>
<td>The USP monograph tests for Assay and Content of (specific constituent or marker) are used to measure the amount of a substance in an ingredient, (which in FDA’s language is the overall purity of the ingredient).</td>
</tr>
<tr>
<td>Composition</td>
<td>Composition applies to multiple constituents in an ingredient.</td>
</tr>
<tr>
<td>Contaminants</td>
<td>Contaminants may arise from any source extraneous to the manufacturing process or may be introduced by contamination or adulteration. Contaminants can be classified as microbiological (e.g., objectionable microorganisms) or chemical (e.g., pesticides). Analytical procedures for the determination of contaminants can be quantitative assays or limit tests.</td>
</tr>
<tr>
<td>Impurities</td>
<td>Impurities may arise from the manufacture (e.g., reagents, byproducts) or storage (e.g., degradation) of an article. Impurities can be classified as organic or inorganic. Setting impurity or degradation product limits for articles is based on chemistry and safety concerns. Analytical procedures for the determination of impurities can be quantitative assays or limit tests.</td>
</tr>
<tr>
<td>Specific Tests</td>
<td>Specific tests are sometimes included to further characterize an article, such as tests for the chemo-physical nature of botanicals: macroscopy and microscopy fall here</td>
</tr>
</tbody>
</table>
Specifications in a USP Dietary Ingredient Monograph

<table>
<thead>
<tr>
<th>USP Term(s)</th>
<th>USP Monograph Test Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Identification</strong></td>
<td>The monograph section titled “Identification” may consist of one or more (orthogonal) tests to verify the identity of articles. Failure of the article to meet one or more of the tests under the identification section indicates that the article is mislabeled and/or adulterated.</td>
</tr>
</tbody>
</table>

Plantain mixed with/substituted with Digitalis in 1998:

- Remember the case of plantain mixed with *Digitalis lanata* in 1998
- The C of A for the raw plant material had a description of certain of its physical characteristics, such as “color and flavor” only no chemical testing.
- If orthogonal test had been used to identify the raw material chances of passing would have been reduced significantly.
It may look right but strength may not be: may be weaker than required thus no beneficial effects—Black cohosh products

But adulterated/ratio of different components
  – Weight loss products
  – Performance enhancement products
  – Sexual enhancement products
Test for flavonoids – HPTLC
Track assignment: 1) USP Rutin RS 0.6 mg/mL, USP Chlorogenic RS 0.2 mg/mL, and USP Quercetin RS (with increasing RF); 2) ginkgo leaves (commercial sample); 3) ginkgo leaves (wild crafted); 4) ginkgo leaves, powder (commercial sample A); 5) ginkgo leaves, powder (commercial sample B); 6) ginkgo leaves, tincture (commercial sample)

Test for terpene lactones – HPTLC
Track assignment: 1) bilobalide (commercial sample), 1 mg/mL; 2) USP Ginkgo Terpene Lactones RS, 10 mg/mL; 3) ginkgo leaves (commercial sample); 4) ginkgo leaves (wild crafted); 5) ginkgo leaves, powder (commercial sample A); 6) ginkgo leaves, powder (commercial sample B)
Left image: Derivatize, heat at 180º for 10 min, cool, and examine under UV light at 254 nm;
Right image: Derivatize, heat at 180º for 10 min, cool, and at 366 nm

Content of flavonol glycosides – HPLC

Ginkgo Terpene Lactones - HPLC

Ginkgolic Acids - HPLC
If the material does not disintegrate you may end up with adverse effects—intestinal obstruction, choking.

Will not get the effect required.

Examples:
- prebiotics such as psyllium, inulin
- vitamins
### Contaminants

| Contaminants | Contaminants may arise from any source extraneous to the manufacturing process or may be introduced by contamination or adulteration. Contaminants can be classified as microbiological (e.g., objectionable microorganisms) or chemical (e.g., pesticides). Analytical procedures for the determination of contaminants can be quantitative assays or limit tests. |

- Microbial contamination – may lead to infection and other adverse effects
- Heavy metals poisoning – examples of some dietary supplements containing heavy metals e.g. botanical DS adopted from Ayurveda medical system have been found in the USA market
Impurities

| Impurities | Impurities may arise from the manufacture (e.g., reagents, byproducts) or storage (e.g., degradation) of an article. Impurities can be classified as organic or inorganic. Setting impurity or degradation product limits for articles is based on chemistry and safety concerns. Analytical procedures for the determination of impurities can be quantitative assays or limit tests. |

- **Creatine**
  - Depending on manufacture method may be contaminated with cancer causing agents such as dihydrotriazine

- **Comfrey**
  - Depending on manufacture method may be contaminated with hepatotoxic pyrrolizidine alkaloids

- **Tryptophan**
  - Depending on manufacture method May be contaminated with EBT that can cause eosinophilia myalgia syndrome (EMS)
Instructions on what goes on the label

To comply with cGMPs

- Ensure safety

  The label of an herb or other botanical intended for use as a dietary supplement (that claim to comply to USP standards) shall bear the statement, “If you are pregnant or nursing a baby, seek the advice of a health professional before using this product.” unless exempt.

- Admission may be pegged to the inclusion of warning statement e.g. for Black cohosh, St Jon’s Wort, Willow Bark
Example of labels to protect public

- **St. Johns Wort:** The label bears a statement indicating that “Rare cases of allergic reactions and photosensitivity have been reported with the use of St. John’s Wort. St. John’s Wort interacts with numerous medications. Check with your healthcare provider before using.”

- **Black Cohosh:** Dosage forms prepared with this article should bear the following statement: Discontinue use and consult a healthcare practitioner if you have a liver disorder or develop symptoms of liver trouble, such as abdominal pain, dark urine, or jaundice.

- **Willow Bark:** The label bears a statement indicating “Not for use in children, women who are pregnant or nursing, or by persons with known sensitivity to aspirin”.
In conclusion

- USP DS quality standards are developed with input from EC (from different disciplines) thus have the best possible information
- Public/stakeholder input sought (PF) and incorporated
- Ensure public receives quality OTC and DS products
- Monograph attributes each designed to protect public health as discussed
- Products that comply with USP quality standards protect public health and in public interest
Thank You