Coix lacryma-jobi Seed

Proposed For Comment Version 0.2

Coix lacryma-jobi Seed

DEFINITION
The article consists of the dried ripe caryopsis, freed from the shell, of Coix lacryma-jobi var. ma-yuen (Rom.Caill.) Stapf (Family Poaceae, alt. Gramineae), collected in the fall. It contains NLT 3.5% of triglycerides calculated as the sum of trilinolein (C_{57}H_{98}O_6), 1,2-dilinoleoyl-3-palmitin (C_{55}H_{96}O_6), 1,2-dilinoleoyl-3-olein (C_{57}H_{100}O_6), 1-palmitoyl-2-oleoyl-3-linolein (C_{55}H_{100}O_6), 1,2-dioleoyl-3-linolein (C_{57}H_{102}O_6), 1,2-dioleoyl-3-palmitin (C_{55}H_{102}O_6), and triolein (C_{57}H_{104}O_6), on the dried basis.

SYNONYMS
Coix chinensis Tod.
Coix chinensis Tod. ex Balansa
Coix ma-yuen Rom.Caill.

POTENTIAL CONFOUNDING MATERIALS
Coix lacryma-jobi L.
Coix puellarum Balansa
Other varities of Coix lacryma-jobi, such as Coix lacryma-jobi var. stenocarpa Olive.
Sorghum bicolor (L.) Moench
Hordeum vulgare L.

SELECTED COMMON NAMES
Chinese: 薏苡仁 (Yi Yi Ren), 薏米 (Yi Mi)
English: Coix seed, Job’s Tears, adlay
French: Larmes de Job
German: Hiobstrâne
Japanese: ジュズダマ
Korean: 율무
Russian: Coix семян, Иовлевы слёзь
Spanish: Lágrimas de Job

CONSTITUENTS OF INTEREST
Triglycerides: Trilinolein, 1,2-dilinoleoyl-3-palmitin, 1,2-dilinoleoyl-3-olein, 1-palmitoyl-2-oleoyl-3-linolein, 1,2-dioleoyl-3-linolein, 1,2-dioleoyl-3-palmitin, and triolein
Fatty acid: Oleic acid
IDENTIFICATION

• A. BOTANICAL CHARACTERISTICS

Macroscopic: Broad ovoid or elongated-elliptical, 4–8 mm long, 3–6 mm wide. Externally milky-white, smooth, occasionally with yellowish-brown testa. One end obtusely rounded, the other end relatively broad and slightly dented with one pale brown dotted hilum. Dorsal surface rounded and protruding; ventral surface with one relatively broad and deep longitudinal furrow. Texture hard, fracture white and starchy.

Microscopic

The Powder: Starch granules numerous and often in clumps, simple granule subround or polyhedral, 2-20 μm in diameter with stellate, y-shaped hilum; compound granule rare, usually consisting of 2-3 simple granules.

• B. THIN-LAYER CHROMATOGRAPHY

Standard solution A: 2 mg/mL of USP Oleic Acid RS in methanol
Standard solution B: 40 mg/mL (about 50 μL/mL) of USP Coix lacryma-jobi Seed Oil Extract RS in methylene chloride
Sample solution: Sonicate 0.5 g of Coix lacryma-jobi Seed, finely powdered, in 5 mL of methylene chloride for 10 min. Centrifuge at 5000 rpm for 5 min and use the supernatant.

Chromatographic system

(See High-Performance Thin-Layer Chromatography Procedure for Identification of Articles of Botanical Origin <203> in First Supplement to USP 38–NF 33, 2015, p. 7044.)

Test conditions and System suitability (see Identification of Fixed Oils by Thin-Layer Chromatography <202> in First Supplement to USP 38–NF 33, 2015, p. 7042).

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 in a saturated chamber (saturated for 20 min with filter paper) up to 7 cm, remove the plate from the chamber, and dry in air. Treat the plate with Spray reagent, heat at 120° for 3 min, and examine in daylight.

Acceptance criteria: The Sample solution exhibits, in the upper half, a band corresponding in Rf and color to the band due to oleic acid in Standard solution A. The Sample solution exhibits additional bands corresponding to similar bands in Standard solution B; these include one band right above oleic acid with the same color as it, a cluster of four blue bands in the lower half, and some faint bands in the middle-third.

• C. HPLC

Analysis: Proceed as directed in the Assay for Content of Triglycerides.

Acceptance criteria: The Sample solution exhibits a peak with a retention time corresponding to triolein in Standard solution A; the peaks due to trilinolein, 1,2-dilinoleoyl-3-palmitin,1,2-dilinoleoyl-3-olein, 1-palmitoyl-2-oleoyl-3-linolein, 1,2-dioleoyl-3-linolein, and 1,2-dioleoyl-3-palmitin are at retention times corresponding to the same triglycerides in Standard solution B and the reference chromatogram provided with the lot of USP Coix lacryma-jobi Seed Oil Extract RS being used. 1,2-dilinoleoyl-3-olein and 1,2-dioleoyl-3-linolein have the most intense peaks; 1-palmitoyl-2-oleoyl-3-linolein and triolein have medium intensity peaks; trilinolein, 1,2-dilinoleoyl-3-palmitin, and 1,2-dioleoyl-3-palmitin have weak intense peaks (distinction from Sorghum bicolor (L) Moench, which has similar triglycerides peaks but all of them are weak intensity; and Hordeum vulgare L., which has four triglycerides peaks and all of them are weak intensity).

ASSAY
**Content of Triglycerides**

**Mobile phase:** Methanol

**Standard solution A:** 0.20 mg/mL of USP Triolein RS in methanol. Sonicate to dissolve.

**Standard solutions:** Dilute Standard solution A with methanol to obtain solutions of 0.0125, 0.025, 0.05, 0.10, and 0.20 mg/mL of USP Triolein RS. Pass each through a membrane filter of 0.45-μm or finer pore size.

**Standard solution B:** 0.5 mg/mL of USP Coix lacryma-jobi Seed Oil Extract RS in methanol

**Sample solution:** Accurately transfer about 500 mg of Coix lacryma-jobi Seed, finely powdered, to a suitable flask and add 35 mL of methanol. Sonicate for 30 min and filter with the aid of vacuum into a vacuum flask. Rinse the flask and the residue left in the flask with 5 mL of methanol, and wash the residue and paper on the filter using the rinsing. Repeat the rinse and wash procedure one more time. Transfer the filtrate into a 50-mL volumetric flask. Wash the vacuum flask with methanol, combine the washing into the volumetric flask, dilute with methanol to volume, and mix. Before injection, pass through a membrane filter of 0.45-μm or finer pore size, and discard the first portion of the filtrate. 

**[Note—The filter and vacuum flask should be dry.]**

**Chromatographic system**

(See Chromatography <621>, System Suitability.)

- **Mode:** LC
- **Detector:** Evaporative light-scattering.  
  [Note—The parameters of the detector are adjusted to achieve the best signal-to-noise ratio, according to manufacturer recommendations.]
- **Column:** 3.0-mm × 10-cm; 2.7-μm packing L7 (similar to AMT HALO™ C8 or Agilent Poroshell 120 EC C8)
- **Column temperature:** 20°
- **Flow rate:** 0.3 mL/min
- **Injection volume:** 5 μL

**System suitability**

- **Samples:** Standard solution A, Standard solutions, and Standard solution B
- **Suitability requirements**
  - **Chromatogram similarity:** The chromatogram of Standard solution B is similar to the reference chromatogram provided with the lot of USP Coix lacryma-jobi Seed Oil Extract RS being used.
  - **Resolution:** NLT 1.5 between the peak of 1,2-dilinoleoyl-3-olein and the small peak following it, Standard solution B
  - **Tailing factor:** NMT 1.5 for triolein peak, Standard solution A
  - **Relative standard deviation:** NMT 5.0% for the triolein peak, Standard solution A
  - **Signal-to-noise ratio:** NLT 15 for the triolein peak, Standard solutions of 0.0125 mg/mL
  - **Correlation coefficient:** NLT 0.995 for the regression line as determined in Analysis, Standard solutions

**Analysis**

- **Samples:** Standard solutions, Standard solution B, and Sample solution

Using the chromatograms of Standard solutions, Standard solution B, and the reference chromatogram provided with the lot of USP Coix lacryma-jobi Seed Oil Extract RS being used, identify the retention times of the peaks corresponding to trilinolein, 1,2-dilinoleoyl-3-palmitin, 1,2-dilinoleoyl-3-olein, 1-palmitoyl-2-oleoyl-3-linolein, 1,2-dioleoyl-3-linolein, 1,2-dioleoyl-3-linolein, 1,2-dioleoyl-3-palmitin, and triolein in the Sample solution.  

[Note—The approximate relative retention times of the analytes are provided in Table 1.]

Table 1
<table>
<thead>
<tr>
<th>Analyte</th>
<th>Approximate Relative Retention Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trilinolein</td>
<td>0.61</td>
</tr>
<tr>
<td>1,2-Dilinoleoyl-3-palmitin</td>
<td>0.67</td>
</tr>
<tr>
<td>1,2-Dilinoleoyl-3-olein</td>
<td>0.71</td>
</tr>
<tr>
<td>1-Palmitoyl-2-oleoyl-3-linolein</td>
<td>0.79</td>
</tr>
<tr>
<td>1,2-Dioleoyl-3-linolein</td>
<td>0.84</td>
</tr>
<tr>
<td>1,2-Dioleoyl-3-palmitin</td>
<td>0.94</td>
</tr>
<tr>
<td>Triolein</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Plot the logarithms of peak responses versus the logarithms of concentrations, in mg/mL, of triolein from the *Standard solutions*, and determine the regression line using a least-squares analysis; or, establish a linear regression equation using a least-squares analysis according to the logarithms of the peak responses versus logarithms of concentrations, in mg/mL, of triolein from the *Standard solutions*. Determine the concentration, $C$, in mg/mL, of the relevant analyte in the *Sample solution* by regression line or linear regression equation.

Separately calculate the percentages of trilinolein, 1,2-dilinoleoyl-3-palmitin, 1,2-dilinoleoyl-3-olein, 1-palmitoyl-2-oleoyl-3-linolein, 1,2-dioleoyl-3-linolein, 1,2-dioleoyl-3-palmitin, and triolein in the portion of *Coix lacryma-jobi* Seed taken:

$$\text{Result} = C \times \left( \frac{V}{W} \right) \times 100$$

$C$ = concentration of the relevant analyte in the *Sample solution* as determined above (mg/mL)

$V$ = volume of the *Sample solution* (mL)

$W$ = weight of *Coix lacryma-jobi* Seed taken to prepare the *Sample solution* (mg)

Add the percentages of trilinolein, 1,2-dilinoleoyl-3-palmitin, 1,2-dilinoleoyl-3-olein, 1-palmitoyl-2-oleoyl-3-linolein, 1,2-dioleoyl-3-linolein, 1,2-dioleoyl-3-palmitin, and triolein.

**Acceptance criteria:** NLT 3.5% on the dried basis

**CONTAMINANTS**

- **Test for Zearalenone**

  **Mobile phase:** Acetonitrile and water (1:1)

  **Standard stock solution:** 250 ng/mL of zearalenone in methanol

  **Standard solution:** Accurately transfer 1 mL of *Standard stock solution* to a 10-mL volumetric flask and add methanol to volume.

  **Sample solution:** Accurately transfer about 20 g of *Coix lacryma-jobi* Seed, finely powdered, to a suitable centrifuge tube. Add 4.0 g of sodium chloride, then accurately add 100 mL of 90% acetonitrile. Mix for 2 min with a high speed disperser (not lower than 11000 rpm), and centrifuge for 5 min (4000 rpm). Immediately pipet 10 mL of the supernatant into a 50-mL volumetric flask, add water to volume, mix, and centrifuge. Pipet 10 mL of the supernatant onto the Immunoaffinity Column (*ZearalaTest™*), at a flow rate of 3 mL/min. Wash the column with 10 mL of water at a flow rate of 6 mL/min, let the column run dry, and discard the water elution. Wash the column with 1.5 mL of methanol at a flow rate of 1 mL/min, collect the methanol elution into a 2-mL volumetric flask, let the column run dry, add methanol
to volume, and mix.

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

**Mode:** LC

**Detector:** Fluorescence detector set at excitation wavelength (Ex) 232 nm and emission wavelength (Em) 460 nm

**Column:** 4.6-mm × 15-cm; 5-µm packing L1 (similar to Inertsil ODS-3)

**Column temperature:** 30°

**Flow rate:** 1.0 mL/min

**Injection volume:** 20 µL of *Sample solution*

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

- **Theoretical plates:** NLT 10000
- **Relative standard deviation:** NMT 10.0% for the zearalenone peak
- **Correlation coefficient:** NLT 0.999 for the regression line as determined in the *Analysis*

**Analysis**

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

Inject the *Standard solution* with volumes of 5, 10, 15, 20, and 25 µL, and separately measure the peak areas of each injection. Plot the peak responses versus the amount, in ng, of zearalenone in the *Standard solution* injections, and determine the regression line using a least-squares analysis. Using the chromatogram of the *Standard solution* identify the retention time of the peak corresponding to zearalenone in the *Sample solution*.

From the graph, determine the content, C, in ng, of zearalenone in the *Sample solution*.

Calculate the content, in ng/g, of zearalenone in the portion of *Coix lacryma-jobi* Seed taken:

\[
\text{Result} = 5000 \times \left(\frac{C}{W}\right)
\]

\(C\) = content of zearalenone as determined above (ng)

\(W\) = weight of *Coix lacryma-jobi* Seed taken to prepare the *Sample solution* (g)

**Acceptance criteria:** NMT 60 ng/g

- **ARTICLES OF BOTANICAL ORIGIN <561>, Limits of Elemental Impurities:** Meets the requirements
- **ARTICLES OF BOTANICAL ORIGIN <561>, General Method for Pesticide Residues Analysis:** Meets the requirements

- **MICROBIAL ENUMERATION TESTS <61>:** 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 bacteria does not exceed \(10^3\) cfu/g.

- **TESTS FOR SPECIFIED MICROORGANISMS <62>:** Meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli*

- **ARTICLES OF BOTANICAL ORIGIN <561>, Aflatoxins:** Meets the requirements

**SPECIFIC TESTS**

- **ARTICLES OF BOTANICAL ORIGIN <561>, Foreign Organic Matter:** NMT 2.0%
- **ARTICLES OF BOTANICAL ORIGIN <561>, Alcohol-Soluble Extractives, Method 1:** Using absolute alcohol, NLT 5.5%
• **Loss on Drying <731>** [7]
  
  **Sample:** 2.0 g of *Coix lacryma-jobi* Seed, finely powdered
  
  **Analysis:** Dry the *Sample* at 105° for 5 h.
  
  **Acceptance criteria:** NMT 15.0%

• **Articles of Botanical Origin <561>, [3]** [3] *Total Ash*
  
  **Analysis:** 2.0 g of *Coix lacryma-jobi* Seed, finely powdered
  
  **Acceptance criteria:** NMT 3.0%

### Additional Requirements

• **Packaging and Storage:** Preserve in well-closed containers, protected from light and moisture, and store at 4°–10°.

• **Labeling:** The label states the Latin binomial and the part(s) of the plant contained in the article.

• **USP Reference Standards <11>** [8]
  
  USP *Aflatoxins* [6] RS
  
  USP *Coix lacryma-jobi* Seed Oil Extract RS
  
  USP *Oleic Acid* in RS
  
  USP Triolein RS

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