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Powdered Soy Isoflavones Extract

DEFINITION

Powdered Soy Isoflavones Extract is prepared from the seeds of *Glycine max* Merr. (Fam. Fabaceae) by extraction with water or hydroalcoholic mixtures. It contains NLT 90.0% and NMT 110.0% of the labeled amount of isoflavones, calculated on the dried basis as the sum of daidzin, glycitin, genistin, and one or more of the following isoflavones: malonyl daidzin, malonyl glycitin, malonyl genistin, acetyl daidzin, acetyl glycitin, acetyl genistin, daidzein, glycitein, and genistein.

IDENTIFICATION

• A. HPLC IDENTIFICATION TEST

Analysis: Proceed as directed in the test for *Content of Isoflavones*.

Acceptance criteria: The retention times of the daidzin, glycitin, and genistin peaks from the *Sample solution* correspond to those of *Standard solutions A–E*.

COMPOSITION

• CONTENT OF ISOFLAVONES

Diluent: Acetonitrile and water (2:3)

Internal standard solution: 2.0 mg/mL [USP Apigenin RS](#) in dimethyl sulfoxide. [NOTE—This solution is stable for 6 months when stored in a tightly closed, light-resistant glass container at room temperature.]

System suitability solution 1: Transfer 1 g of [USP Defatted Powdered Soy RS](#) to a centrifuge tube, fitted with PTFE or polyethylene-lined screw caps. Add the following in exact volumes: 0.5 mL of *Internal standard solution*, 10 mL of acetonitrile (swirl to disperse), and 6.0 mL of water. Cap, shake on an orbital or wrist-action shaker for 60 min, add 8.5 mL of water, and centrifuge. Pass a portion of the supernatant through a hydrophilic propylene or PVDF membrane having a 0.45-μm or finer pore size, discarding the first 5 mL of filtrate.

System suitability solution 2: Heat 1 g of [USP Defatted Powdered Soy RS](#) in a shallow porcelain dish at 120° for 120 min, and transfer to a centrifuge tube, fitted with PTFE or polyethylene-lined screw caps. Add the following in exact volumes: 0.5 mL of *Internal standard solution*, 10 mL of acetonitrile (swirl to disperse), and 6.0 mL of water. Cap, shake on an orbital or wrist-action shaker for 60 min, add 8.5 mL of water, and centrifuge. Pass a portion of the supernatant through a hydrophilic propylene or PVDF membrane having a 0.45-μm or finer pore size, discarding the first 5 mL of filtrate.

[NOTE—*Standard stock solution* and *Standard solutions A–E* are stable for 2 months when stored in a tightly closed, light-resistant glass container at room temperature.]

Standard stock solution: Contains the following in dimethyl sulfoxide: 2.0 mg/mL of [USP Daidzin RS](#), 0.5 mg/mL of [USP Glycitin RS](#), 2.0 mg/mL of [USP Genistin RS](#), 0.2 mg/mL of [USP Daidzein RS](#), 0.2 mg/mL of [USP Glycitein RS](#), and 0.2 mg/mL of [USP Genistein RS](#)

Standard solution A: Add 0.5 mL of *Standard stock solution* and 0.5 mL of *Internal standard solution* to a 25-mL volumetric flask, and dilute with *Diluent* to volume.

Standard solution B: Add 1.0 mL of *Standard stock solution* and 0.5 mL of *Internal standard solution* to a 25-mL volumetric flask, and dilute with *Diluent* to volume.

Standard solution C: Add 1.5 mL of *Standard stock solution* and 0.5 mL of *Internal standard solution* to a 25-mL volumetric flask, and dilute with *Diluent* to volume.

Standard solution D: Add 2.0 mL of *Standard stock solution* and 0.5 mL of *Internal standard solution* to a 25-mL volumetric flask, and dilute with *Diluent* to volume.

Standard solution E: Add 2.5 mL of *Standard stock solution* and 0.5 mL of *Internal standard solution* to a 25-mL volumetric flask, and dilute with *Diluent* to volume.

Sample solution: Transfer a quantity of Powdered Soy Isoflavones Extract, equivalent to NMT 5 mg of isoflavones, to a 30-mL glass centrifuge tube, fitted with a PTFE or polyethylene-lined screw cap. Add the following in exact volumes: 0.5 mL of *Internal standard solution*, 10 mL of acetonitrile (swirl to disperse), and 6.0 mL of water. Cap, shake on an orbital or wrist-action shaker for 60 min, add 8.5 mL of water, and centrifuge. Pass a portion of the supernatant through a hydrophilic propylene or PVDF membrane having a 0.45-μm or finer pore

size, discarding the first 5 mL of filtrate. [NOTE—Do not use nylon filters. Analyze samples containing significant amounts of acetyl and/or malonyl isoflavones within 4 h of preparation.]

Solution A: 0.05% phosphoric acid in water

Solution B: Acetonitrile

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

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
60	70	30
60.5	10	90
63.5	10	90
64	90	10
74	90	10

Chromatographic system

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

Mode: LC

Detector: UV 260 nm

Column: 3.0-mm × 25-cm; 5-μm packing L1

Column temperature: 40°

Flow rate: 0.65 mL/min

Injection size: 5 μL

[NOTE—System suitability 1 and System suitability 2 must both be met.]

System suitability 1

Sample: Standard solution C

Suitability requirements

Chromatogram similarity: The chromatogram from Standard solution C is similar to the reference chromatogram provided with the lot of USP reference standard being used for the isoflavones mentioned.

Tailing factor: NLT 0.8 and NMT 1.2 for the daidzin peak

Relative standard deviation: NMT 2.0% for the genistin peak

System suitability 2

Samples: System suitability solution 1 and System suitability solution 2

Suitability requirements

Chromatogram similarity: The chromatograms from System suitability solution 1 and System suitability solution 2 are similar to those provided with the lot of [USP Defatted Powdered Soy RS](#) being used.

Resolution: NLT 1.0 between the acetyl glycitin and malonyl genistin peaks, and NLT 2.0 between any other consecutive pair of isoflavone peaks

Analysis

Samples: Standard solutions A–E and Sample solution

Measure the peak areas of the analytes and the internal standard. Determine the ratio of the peak areas of each analyte to the internal standard peak area. Plot the ratios of the relevant peak responses versus the concentrations, in mg/mL, of each analyte obtained from the Standard solutions A–E, and determine the regression line by least-squares analysis. The correlation coefficient for each of the regression lines is NLT 0.999. From the graphs so obtained, determine the concentration, C, in mg/mL, of the relevant analyte in the Sample solution.

Separately calculate the percentages of daidzin, glycitin, and genistin, and of daidzein, glycitein, and genistein, if present, in the portion of Powdered Soy Isoflavones Extract taken:

$$\text{Result} = (C/W) \times V \times 100$$