

Status: Currently Official on 16-Feb-2025
 Official Date: Official Prior to 2013
 Document Type: Dietary Supplement Monographs
 DocId: GUID-683A08A4-E4C7-4DB4-A080-B97DFF5500C3_1_en-US
 DOI: https://doi.org/10.31003/USPNF_M1454_01_01
 DOI Ref: jq1hg

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Soy Isoflavones Capsules

DEFINITION

Soy Isoflavones Capsules contain Powdered Soy Isoflavones Extract. Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of Extract, represented by the sum of the content of the isoflavones 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

The retention times of the daidzin, glycitin, and genistin peaks in the chromatogram of the *Sample solution* correspond to those of *Standard solutions A–E*, as obtained in the test for *Content of Isoflavones*.

STRENGTH

• CONTENT OF ISOFLAVONES

Diluent: Acetonitrile and water (2:3)

Internal standard solution: 2.0 mg/mL of [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.]

Malonyl/Acetyl isoflavones retention times check solution 1: 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 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, mix, and centrifuge. Pass a portion of the supernatant through a hydrophilic propylene or PVDF membrane of 0.45-µm or finer pore size, discarding the first 5 mL of filtrate.

Malonyl/Acetyl isoflavones retention times check solution 2: Transfer 1 g of [USP Defatted Powdered Soy RS](#) to a centrifuge tube, fitted with 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, mix, and centrifuge. Pass a portion of the supernatant through a hydrophilic propylene or PVDF membrane of 0.45-µm or finer pore size, discarding the first 5 mL of filtrate.

[NOTE—All *Standard solutions* and *Standard stock solutions* 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](#), and 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

Hard-gelatin Capsules: Weigh and finely powder the content of NLT 20 Capsules. Transfer an accurately weighed quantity of the powder, equivalent to NMT 5 mg of isoflavones, to a suitable glass centrifuge tube, fitted with PTFE or polyethylene-lined screw cap.

Soft-gelatin Capsules: Weigh and homogenize NLT 20 Capsules. Transfer an accurately weighed quantity of the mixture, equivalent to NMT 5 mg of isoflavones, to a suitable glass centrifuge tube, fitted with PTFE or polyethylene-lined screw cap.

Add the following in exact volumes to the centrifuge tube: 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, mix, and centrifuge. Pass a portion

of the supernatant through a hydrophilic propylene or PVDF membrane of 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 the gradient table below.

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

System suitability

Samples: *Standard solution C, Malonyl/Acetyl isoflavones retention times check solution 1, and Malonyl/Acetyl isoflavones retention times check solution 2*

Suitability requirements

Chromatogram similarity: The chromatograms obtained from *Standard solution C, Malonyl/Acetyl isoflavones retention times check solution 1, and Malonyl/Acetyl isoflavones retention times check solution 2* are similar to the Reference chromatograms provided with the [USP Defatted Powdered Soy RS](#).

Tailing factor: NLT 0.8 and NMT 1.2 for the daidzin peak, *Standard solution C*

Relative standard deviation: NMT 2.0%, for the genistin peak, in repeated injections, *Standard solution C*

Resolution: NLT 1.0 between acetyl glycitin and malonyl genistin, and NLT 2.0 between any other consecutive pair of isoflavone peaks, *Malonyl/Acetyl isoflavones retention times check solution 2*

Analysis

Samples: *Standard solution A, Standard solution B, Standard solution C, Standard solution D, Standard solution E, Malonyl/Acetyl isoflavones retention times check solution 1, Malonyl/Acetyl isoflavones retention times check solution 2, and Sample solution*

Identify the peaks of daidzin, glycitin, genistin, daidzein, glycitein, and genistein in the chromatograms of *Standard solutions A–E* by comparison with the Reference Standard Chromatogram provided with the appropriate USP Reference Standard used. Measure the peak areas of the analytes and the internal standard peaks. Determine the ratio of the peak areas of each analyte to the internal standard peak areas. Plot the ratios of the relevant peak responses versus the concentrations, in mg/mL, of each analyte obtained from *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 obtained, determine the concentration, C, in mg/mL, of daidzin, glycitin, and genistin, in addition to daidzein, glycitein, and genistein, if present, in the *Sample solution*. Identify the peaks of malonyl daidzin, malonyl glycitin, acetyl daidzin, acetyl glycitin, malonyl genistin, and acetyl genistin in the chromatograms of the *Malonyl/Acetyl isoflavones retention times check solutions* by comparison with the Reference Chromatograms provided with the [USP Defatted Powdered Soy RS](#). From the graphs obtained for daidzin, glycitin and genistin, determine the concentration, C, in mg/mL, of the corresponding malonyl and acetyl derivatives, if present, in the *Sample solution*:

$$\text{Result} = C_0 \times F$$