

Powdered Soy Isoflavones Extract

Powdered Soy Isoflavones Extract is prepared from the seeds of *Glycine max* Merr. (Fam. Fabaceae) by extraction with water or hydroalcoholic mixtures. It contains not less than 90.0 percent and not more than 110.0 percent 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.

Packaging and storage— Preserve in tight, light-resistant containers, and store at controlled room temperature.

Labeling— The label states the Latin binomial and, following the official name, the part of the plant from which the article was prepared. The label also indicates the content of isoflavones. It meets other requirements for labeling under [Botanical Extracts](#) 565 .

Identification— The retention times of the daidzin, glycitin, and genistin peaks in the chromatogram of the Test solution correspond to those in the chromatogram of the Working standard solutions, as obtained in the test for Content of isoflavones.

[Microbial enumeration](#) 2021 — The total aerobic microbial count does not exceed 10⁴ cfu per g, and the total combined molds and yeasts count does not exceed 10³ cfu per g.

Absence of specified microorganisms 2022 — It meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*.

[Loss on drying](#) 731 — Dry about 1.0 g of the Powdered Soy Isoflavones Extract, accurately weighed, at 130°C or 2 hours: it loses not more than 7.0% of its weight.

Aflatoxins 561 : meets the requirements.

[Heavy metals, Method II](#) 231 : not more than 10 µg per g.

Content of isoflavones—

Diluting solution— Prepare a mixture of acetonitrile and water(4:6).

Internal standard solution— Quantitatively dissolve an accurately weighed quantity of USP Apigenin RS in dimethyl sulfoxide to obtain a solution having a concentration of about 2.0 mg per

mL. [Note—The solution is stable for 6 months when stored in a tightly closed, light-resistant glass container at room temperature.]

Solution A— Prepare filtered and degassed water containing 0.05% phosphoric acid.

Solution B— Use filtered and degassed acetonitrile.

Mobile phase— Use variable mixtures of Solution A and Solution B as directed for Chromatographic system. Make adjustments if necessary.

Standard stock solution— Dissolve accurately weighed quantities of USP Daidzin RS, USP Glycitin RS, USP Genistin RS, USP Daidzein RS, USP Glycitein RS, and USP Genistein RS in dimethyl sulfoxide to obtain a solution having known concentrations of about 2.0, 0.5, 2.0, 0.2, 0.2, 0.2 mg per mL, respectively. [Note—The solution is stable for 2 months when stored in a tightly closed, light-resistant glass container at room temperature.].

Working standard solutions— Transfer 0.5, 1.0, 1.5, 2.0, and 2.5 mL of the Standard stock solution to separate 25-mL volumetric flasks, add 0.5 mL of the Internal standard solution to each flask, and dilute quantitatively with Diluting solution to obtain Working standard solutions 1, 2, 3, 4, and 5, having known concentrations of the six USP Reference Standards as listed in the table. [Note—The solutions are stable for 2 months when stored in tightly closed, light-resistant glass containers at room temperature.]

Working standard solution	Daidzin (mg/mL)	Glycitin (mg/mL)	Genistin (mg/mL)	Daidzein (mg/mL)	Glycitein (mg/mL)	Genistein (mg/mL)	Apigenin (mg/mL)
1	0.040	0.010	0.040	0.004	0.004	0.004	0.040
2	0.080	0.020	0.080	0.008	0.008	0.008	0.040
3	0.120	0.030	0.120	0.012	0.012	0.012	0.040
4	0.160	0.040	0.160	0.016	0.016	0.016	0.040
5	0.200	0.050	0.200	0.020	0.020	0.020	0.040

Test solution— Transfer an accurately weighed quantity of Powdered Soy Isoflavones Extract, equivalent to not more than 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 minutes, add 8.5 mL of water, mix, and centrifuge. Pass a portion of the supernatant through a hydrophilic propylene or a PVDF membrane having a 0.45- μ m or finer porosity, discarding the first 5 mL of the filtrate. [Notes—Do not use nylon

filters. Analyze samples containing significant amounts of acetyl and/or malonyl isoflavones within 4 hours of preparation.]

Malonyl/acetyl isoflavones retention times check solutions— Heat a 1-g portion of USP Defatted Powdered Soy RS in a shallow porcelain dish at 120 °C for 120 minutes. Transfer another 1-g portion of USP Defatted Powdered Soy RS and the heated portion to two separate 30-mL glass centrifuge tubes, fitted with PTFE or polyethylene-lined screw caps. Proceed as directed for Test solution, starting with “Add the following in exact volumes: 0.5 mL of Internal standard solution,”.

Chromatographic system— The liquid chromatograph is equipped with a 260-nm detector and a 3.0-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 0.65 mL per minute. The column temperature is maintained at 40 °C. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–60	90→70	10→30	linear gradient
60–60.5	70→10	30→90	linear gradient
60.5–63.5	10	90	isocratic
63.5–64	10→90	90→10	linear gradient
64–74	90	10	isocratic

Chromatograph Working standard solution 3, and record the peak responses as directed for Procedure: the chromatogram obtained is similar to the Reference Chromatogram provided with the various USP soy isoflavones Reference Standards appearing in this monograph; the daidzin peak is symmetrical, and the tailing factor is not less than 0.8 and not more than 1.2; and the relative standard deviation, determined from the genistin peak for replicate injections, is not more than 2.0%. Chromatograph the Malonyl/acetyl isoflavones retention times check solutions, and record the peak responses as directed for Procedure: the chromatograms obtained for the heated and unheated check solutions are similar to the Reference Chromatograms provided with USP Defatted Powdered Soy RS; the resolution, R, between any two consecutive isoflavone peaks, with the exception of the acetyl glycitin and malonyl genistin peaks, is not less than 2.0; and the resolution, R, between the acetyl glycitin and the malonyl genistin peaks is not less than 1.0.

Procedure— Separately inject equal volumes (about 5 μL) of the Working standard solutions, the two Malonyl/acetyl Isoflavones retention times check solutions, and the Test solution into the chromatograph; record the chromatograms; and identify the peaks of daidzin, glycitin, genistin, daidzein, glycitein, and genistein in the chromatograms of the Working standard solutions by comparison with the Reference Standard Chromatogram. 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 per mL, of each analyte obtained from the Working standard solutions, and determine the regression line by least-squares analysis. The correlation coefficient for each of the regression lines is not less than 0.999. From the graphs so obtained, determine the concentration, C, in mg per mL, of the relevant analyte in the Test 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 by the formula:

$$2500(C/W)$$

in which C is as obtained above; and W is the weight, in mg, of Powdered Soy Isoflavones Extract taken to prepare the Test 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 USP Defatted Powdered Soy RS. From the graphs obtained for daidzin, glycitin, and genistin, determine the corresponding concentration, C, in mg per mL, of the malonyl and acetyl derivatives, if present, in the Test solution. Separately calculate the percentages of malonyl daidzin, acetyl daidzin, malonyl glycitin, acetyl glycitin, malonyl genistin, and acetyl genistin in the portion of Powdered Soy Isoflavones Extract taken by the formula:

$$2500F(C/W)$$

in which F is the conversion factor for each analyte (1.207 for malonyl daidzin, 1.101 for acetyl daidzin, 1.193 for malonyl glycitin, 1.094 for acetyl glycitin, 1.199 for malonyl genistin, and 1.097 for acetyl genistin); C is as obtained above; and W is the weight, in mg, of Powdered Soy Isoflavones Extract taken to prepare the Test solution. Calculate the content, in percentage, of isoflavones in the portion of the Powdered Soy Isoflavones Extract taken by adding the percentages calculated for all analytes present.

Other requirements— It meets the requirements for Residual Solvents and for Pesticide Residues under [Botanical Extracts](#) [565](#) . USP32