Powdered Ginkgo Extract

» Powdered Ginkgo Extract is prepared from dried and comminuted leaves of Ginkgo extracted with an acetone–water mixture or other suitable solvents. The ratio of the crude plant material to Powdered Extract is between 35 : 1 and 67 : 1. It contains not less than 22.0 percent and not more than 27.0 percent of flavonoids, calculated as flavonol glycosides, with a mean molecular mass of 756.7. It contains not less than 5.4 percent and not more than 12.0 percent of terpene lactones, consisting of between 2.6 percent and 5.8 percent of bilobalide ($C_{15}H_{18}O_8$) and between 2.8 percent and 6.2 percent of ginkgolide A ($C_{20}H_{24}O_9$), ginkgolide B ($C_{20}H_{24}O_{10}$), and ginkgolide C ($C_{20}H_{24}O_{11}$).

Packaging and storage—Preserve in tight, light-resistant containers, protected from moisture, 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 flavonol glycosides and of terpene lactones, the extracting solvent used for preparation, and the ratio of the starting crude plant material to the Powdered Extract.

USP Reference standards ⟨11⟩**—***USP Chlorogenic Acid RS. USP Ginkgo Terpene Lactones RS. USP Ginkgolic Acids RS. USP Quercetin RS. USP Rutin RS.*

Identification—

A: *Thin-Layer Chromatographic Identification Test* ⟨201⟩—

Adsorbent: 0.5-mm layer of chromatographic silica gel mixture.

Test solution—Dissolve about 50 mg of Powdered Extract, accurately weighed, in 10 mL of a mixture of methanol and water (8 : 2). Apply 20 µL.

Standard solution—Prepare a solution of USP Rutin RS and USP Chlorogenic Acid RS in methanol containing 0.3 mg per mL and 0.1 mg per mL, respectively. Apply 10 µL.

Developing solvent system: a mixture of ethyl acetate, water, glacial acetic acid, and anhydrous formic acid (67.5 : 17.5 : 7.5 : 7.5).

Spray reagent 1—Prepare a solution of 2-aminoethyl diphenylborinate in methanol containing 10 mg per mL.

Spray reagent 2—Prepare a solution of polyethylene glycol 4000 in methanol containing 50 mg per mL.

Procedure—Proceed as directed in the chapter, except to dry the plate between 100° and 105°, spray with *Spray reagent 1* while the plate is still warm, and then spray with *Spray reagent 2*. After about 30 minutes, examine the plate under UV light at 365 nm: the chromatogram of the *Standard solution* exhibits a yellow-brown fluorescent zone due to rutin in the lower section and a light blue fluorescent zone due to chlorogenic acid in the middle section. The chromatogram of the *Test solution* exhibits three yellow-brown to greenish fluorescent zones at R_F values below that for the rutin zone in the chromatogram of the *Standard solution;* a green-blue fluorescent zone at an R_F value just above that for the rutin zone in the chromatogram of the *Standard solution;* an intense, light blue fluorescent zone at an R_F value about equal to that of the chlorogenic acid zone in the chromatogram of the *Standard solution;* and two yellow-brown to greenish fluorescent zones in the upper third of the plate. Other, less intense zones may be observed in the chromatogram of the *Test solution.*

B: Proceed as directed in the test for *Content of flavonol glycosides:* the retention times of the peaks for quercetin, isorhamnetin, and kaempferol in the chromatogram of the *Test solution* correspond to those in the chromatogram of the *Standard solution;* the peak for kaempferol is between 0.8 and 1.2 times the size of the quercetin peak; and the peak for isorhamnetin is not less than 0.1 times the size of the quercetin peak.

Microbial enumeration ⟨2021⟩**—**It meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli.* The total aerobic bacterial count does not exceed 10^4 cfu per g, and the total combined molds and yeasts count does not exceed 10^3 cfu per g.

Loss on drying ⟨731⟩**—**Dry about 1.0 g of Powdered Extract, accurately weighed, at 105° for 2 hours: it loses not more than 5.0% of its weight.

Pesticide residues ⟨561⟩**:** meets the requirements.

Change to read:

Limit of ginkgolic acids—

Solution A—Prepare a solution of 0.01% phosphoric acid in water.

Solution B—Prepare a solution of 0.01% phosphoric acid in acetonitrile.

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system.* Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard solution—Dissolve an accurately weighed quantity of USP Ginkgolic Acids RS in methanol, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 0.25 µg per mL of ginkgolic acids, calculated as the sum of the congeners ginkgolic acid C 13 : 0, ginkgolic acid C 15 : 1, and ginkgolic acid C 17 : 1. (RB 1-Apr-2009)

Test solution—Transfer about 0.5 g of Powdered Extract, accurately weighed, to a 10-mL volumetric flask, add 8 mL of methanol to dissolve, and dilute with water to volume.

Chromatographic system (see *Chromatography* ⟨621⟩)— The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm × 5-cm column that contains base-deactivated packing L7. The column temperature is maintained at 35°. The flow rate is about 1.0 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0–6	25→10	75→90	linear gradient
6–7	10	90	isocratic
7–8	10→25	90→75	linear gradient
8–10	25	75	isocratic

Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the chromatogram obtained is similar to the Reference Chromatogram provided with USP Ginkgolic Acids RS; the tailing factor for the ginkgolic acid C 15 : 1 peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 5.0%. (RB 1-Apr-2009) [NOTE—If deterioration of peak shapes is observed, wash the column using a mixture of methanol and water (9 : 1) for 30 minutes.]

Procedure—Separately inject equal volumes (about 100 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, identify the peaks of the relevant analytes by comparison with the Reference Chromatogram, and measure the areas of the ginkgolic acid C 13 : 0, ginkgolic acid C 15 : 1, and ginkgolic acid C 17 : 1 peaks. (RB 1-Apr-2009) Calculate the concentration, in µg per g, of each ginkgolic acid in the portion of Powdered Extract taken by the formula: Calculate the total amount

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of ginkgolic acids by adding the individual contents: the limit is 5 µg per g.

$$\bullet 10(C/W)(r_U / r_S)$$

in which C is the concentration, in µg per mL, of the relevant ginkgolic acid in the *Standard solution*; W is the weight, in g, of Powdered Extract taken to prepare the *Test solution;* and r_U and r_S are the peak areas for the relevant ginkgolic acid obtained from the *Test solution* and the *Standard solution*, respectively.• (RB 1-Apr-2009)

Content of flavonol glycosides—

Extraction solvent, *Mobile phase*, and *Chromatographic system*—Proceed as directed in the test for *Content of flavonol glycosides* under *Ginkgo*.

Standard solutions—Transfer accurately weighed quantities of USP Quercetin RS, kaempferol, and isorhamnetin to separate volumetric flasks, dissolve each in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain *Standard solutions 1*, *2*, and *3* having known concentrations of 0.125 mg per mL, 0.125 mg per mL, and 0.03 mg per mL, respectively.

Test solution—Transfer about 0.300 g of Powdered Extract, accurately weighed, to a 250-mL flask fitted with a reflux condenser. Add 78 mL of *Extraction solvent*, and reflux on a hot water bath for 135 minutes. [NOTE—The solution will turn deep red. The color of the solution is not a definitive indication of reaction completeness.] Allow to cool at room temperature. Transfer to a 100-mL volumetric flask, add water to volume, and mix.

Procedure—Proceed as directed in the test for *Content of flavonol glycosides* under *Ginkgo*. Calculate the percentage of each flavonol glycoside in the portion of Powdered Extract taken by the formula:

$$10(2.51)(C/W)(r_U / r_S)$$

in which W is the weight, in g, of Powdered Extract taken to prepare the *Test solution;* and the other terms are as defined therein. Calculate the total percentage of flavonol glycosides by adding the individual percentages calculated.

Content of terpene lactones—

Solution A, *Solution B*, *Mobile phase*, *Standard solutions*, *Buffer solution*, *Diluent*, and *Chromatographic system*—Proceed as directed in the test for *Content of terpene lactones* under *Ginkgo*.

Test solution—Transfer about 120 mg of Powdered Extract, accurately weighed, to a 25-mL beaker, and proceed as directed for *Test solution* in the test for *Content of terpene lactones* under *Ginkgo*, starting with "add 10 mL of *Buffer solution*", except to dissolve the residue in 20.0 mL of *Diluent*.

Procedure—Proceed as directed in the test for *Content of terpene lactones* under *Ginkgo*. Separately calculate the percentages of bilobalide ($C_{15}H_{18}O_8$), ginkgolide A ($C_{20}H_{24}O_9$), ginkgolide B ($C_{20}H_{24}O_{10}$), and ginkgolide C ($C_{20}H_{24}O_{11}$) in the portion of Powdered Extract taken by the formula:

$$2000(C/W)$$

in which C is the concentration, in mg per mL, of the relevant analyte in the *Test solution;* and W is the weight, in mg, of Powdered Extract taken to prepare the *Test solution*. Calculate the total percentage of terpene lactones in the portion of Powdered Extract taken by adding the percentages calculated for each analyte.

Other requirements—It meets the requirements for *Residue on Evaporation*, *Residual Solvents*, and *Heavy Metals* under *Botanical Extracts* ⟨565⟩.

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