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Powdered Ginkgo Extract

DEFINITION

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 NLT 22.0% and NMT 27.0% of flavonoids, calculated as flavonol glycosides, with a mean molecular mass of 756.7, on the dried basis. It contains NLT 5.4% and NMT 12.0% of terpene lactones, consisting of between 2.6% and 5.8% of bilobalide ($C_{15}H_{18}O_8$) and between 2.8% and 6.2% of the sum of ginkgolide A ($C_{20}H_{24}O_9$), ginkgolide B ($C_{20}H_{24}O_{10}$), and ginkgolide C ($C_{20}H_{24}O_{11}$), on the dried basis.

IDENTIFICATION

• A. [THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST \(201\)](#)

Test for flavanoids

Standard solution: A solution of 0.6 mg/mL of [USP Rutin RS](#) and 0.2 mg/mL each of [USP Chlorogenic Acid RS](#) and [USP Quercetin RS](#) in methanol

Sample solution: 5 mg/mL of Powdered Extract in a mixture of methanol and water (4:1)

Adsorbent: Chromatographic silica gel mixture with an average particle size of 5 µm (HPTLC plates)

Application volume: 5 µL

Developing solvent system: Ethyl acetate, water, anhydrous formic acid, and glacial acetic acid (100:26:11:11)

Spray reagent 1: 5 mg/mL of 2-aminoethyl diphenylborinate in methanol

Spray reagent 2: 50 mg/mL of polyethylene glycol 400 in alcohol

Analysis

Samples: *Standard solution and Sample solution*

Before development of the chromatograms, saturate the chamber for 20 min with *Developing solvent system*. Record temperature and humidity in the laboratory. If the relative humidity exceeds 50%, condition the plate to about 35% relative humidity using a suitable device. Apply the samples separately as bands to a suitable thin-layer chromatographic plate (see [Chromatography \(621\)](#)), and allow the bands to dry. Develop the plate over a path of 6 cm, remove the plate from the chromatographic chamber, and dry in a circulating air oven at 105° for 5 min. Immediately spray the hot plate with *Spray reagent 1*, then with *Spray reagent 2*. Dry, and examine under long-wavelength UV light.

Acceptance criteria: The *Standard solution* shows in its lower part with increasing R_F values a yellowish-brown fluorescent zone due to rutin (R_F 0.28), a light blue fluorescent zone due to chlorogenic acid (R_F 0.36), and a yellow fluorescent zone due to quercetin (R_F 0.92). The *Sample solution* shows a yellowish-brown fluorescent zone, a light blue fluorescent zone, and a yellowish-brown fluorescent zone at R_F similar to those of rutin, chlorogenic acid, and quercetin, respectively, in the *Standard solution*. Additional yellowish to yellowish-green zones due to flavonoids detected in the *Sample solution* chromatogram include one zone below the rutin zone, two zones between the rutin and chlorogenic acid zones, and two zones above the chlorogenic acid zone. Other zones may be seen in the *Sample solution* chromatogram.

• **B. HPLC:** In the test for *Content of Flavonol Glycosides*, the retention times of the peaks for quercetin, isorhamnetin, and kaempferol of the *Sample solution* correspond to those of the *Standard solution*. In the chromatogram of the *Sample solution*, the ratio of the kaempferol peak to the quercetin peak is NLT 0.7, and the peak for isorhamnetin is NLT 0.1 times the size of the quercetin peak.

COMPOSITION

• CONTENT OF FLAVONOL GLYCOSIDES

Extraction solvent: Alcohol, hydrochloric acid, and water (25:4:10)

Mobile phase: Methanol, water, and phosphoric acid (100:100:1)

Standard solution A: 0.125 mg/mL of [USP Quercetin RS](#) in methanol

Standard solution B: 0.125 mg/mL of [USP Kaempferol RS](#) in methanol

Standard solution C: 0.03 mg/mL of [USP Isorhamnetin RS](#) in methanol

Sample solution: Transfer about 0.3 g of Powdered Extract, accurately weighed, to a 250-mL flask fitted with a reflux condenser. Add 78 mL of *Extraction solvent*, and reflux in a hot water bath for 135 min. [NOTE—The solution will turn deep red. The color of the solution is not a definitive indication of reaction completeness.] Allow to cool to room temperature. Transfer to a 100-mL volumetric flask, dilute with water to volume, and mix.

Chromatographic system

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

Mode: LC**Detector:** UV 370 nm**Column:** 4.6-mm × 25-cm; packing L1**Flow rate:** 1.5 mL/min**Injection volume:** 20 µL**System suitability****Samples:** *Standard solution A, Standard solution B, and Standard solution C*[NOTE—The relative retention times for quercetin, kaempferol, and isorhamnetin are about 1.0, 1.8, and 2.0, respectively; *Standard solution A, Standard solution B, and Standard solution C.*]**Suitability requirements****Relative standard deviation:** NMT 2.0% determined from the quercetin peak in repeated injections, *Standard solution A***Analysis****Samples:** *Standard solution A, Standard solution B, Standard solution C, and Sample solution*

Calculate the percentage of each flavonol glycoside in the portion of Powdered Extract taken:

$$\text{Result} = (r_U/r_S) \times (C_S/W) \times F \times 10$$

 r_U = peak area of the relevant analyte from the *Sample solution* r_S = peak area of the relevant analyte from *Standard solution A, Standard solution B, or Standard solution C* C_S = concentration of the relevant analyte in *Standard solution A, Standard solution B, or Standard solution C* (mg/mL) W = weight of Powdered Extract taken to prepare the *Sample solution* (g) F = mean molecular mass factor to convert each analyte into flavonol glycoside with a mean molecular mass of 756.7: 2.504 for quercetin, 2.437 for isorhamnetin, and 2.588 for kaempferol

Calculate the total percentage of flavonol glycosides by adding the individual percentages calculated.

Acceptance criteria: 22.0%–27.0% of flavonoids, calculated as flavonol glycosides with a mean molecular mass of 756.7, on the dried basis**• CONTENT OF TERPENE LACTONES****Solvent:** Methanol and water (9:1)**Buffer solution:** Dissolve 1.19 g of dibasic sodium phosphate and 8.25 g of monobasic potassium phosphate in 1000 mL of water, and adjust to a pH of 5.8.**Diluent:** Methanol and water (1:1)**Solution A:** Water**Solution B:** Methanol**Mobile phase:** See [Table 1](#).**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	75	25
23	52	48
28	52	48
30	25	75
35	10	90
40	75	25
50	75	25

Standard solutions: Using the labeled content of the individual terpene lactones, prepare five solutions of the [USP Ginkgo Terpene Lactones RS](#) in *Diluent* within the range of 5–500 µg/mL for each of the relevant terpene lactones. Use sonication to dissolve the analytes if necessary. Pass through a filter of 0.45-µm or finer pore size.**Sample solution:** Transfer about 120 mg of Powdered Extract, accurately weighed, to a 25-mL beaker. Add 10 mL of *Buffer solution* to the residue, and sonicate for 5 min. Quantitatively transfer the solution to a glass chromatographic tube filled with chromatographic siliceous earth capable of holding 20 mL of aqueous phase.¹ Rinse the beaker with two 5-mL portions of *Buffer solution*, and transfer the washings to the column. Do not exceed 20 mL of the total aqueous phase or the holding capacity of the chromatographic tube. Allow the *Buffer solution*