Ginkgo Capsules

DEFINITION
Ginkgo Capsules are prepared with Powdered Ginkgo Extract and contain, in the labeled amount of Powdered Extract, NLT 22.0% and NMT 27.0% of flavonol glycosides and NLT 5.4% and NMT 12.0% of terpene lactones, calculated as the sum of bilobalide (C₁₉H₂₂O₆), ginkgolide A (C₂₀H₂₄O₉), ginkgolide B (C₂₀H₂₄O₁₀), and ginkgolide C (C₂₀H₂₂O₁₁).

IDENTIFICATION
Change to read:

- **A. 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.
- **B. HPLC:** The retention times of the peaks for bilobalide, ginkgolide A, ginkgolide B, and ginkgolide C of the Sample solution correspond to those of the Standard solutions, as obtained in the test for Content of Terpene Lactones.

STRENGTH
Change to read:

- **CONTENT OF FLAVONOL GLYCOSIDES**
  - **Mobile phase:** Methanol, water, and phosphoric acid (100:100:1)
  - **Standard solution A:** 0.2 mg/mL of USP Quercetin RS in methanol
  - **Standard solution B:** 0.2 mg/mL of USP Kaempferol RS in methanol
  - **Standard solution C:** 0.05 mg/mL of USP Isorhamnetin RS in methanol
  - **Sample solution:** Weigh and finely powder the contents of NLT 20 Capsules. Transfer an accurately weighed quantity of the powder, equivalent to about 50 mg of flavonol glycosides, to a 50-mL volumetric flask. Add 20 mL of methanol, and sonicate for 3 min. Add 20 mL of 1.5 N hydrochloric acid, and sonicate again for 10 min. Allow to cool to room temperature, and dilute with methanol to volume. Centrifuge, and transfer a portion of the clear supernatant to a rubber-capped, low-actinic 30-mL glass centrifuge tube and contain, in the labeled amount of Powdered Extract, NLT 22.0% and NMT 27.0% of flavonol glycosides and NLT 5.4% and NMT 12.0% of terpene lactones, calculated as the sum of bilobalide (C₁₉H₂₂O₆), ginkgolide A (C₂₀H₂₄O₉), ginkgolide B (C₂₀H₂₄O₁₀), and ginkgolide C (C₂₀H₂₂O₁₁).

- **B. HPLC:** The retention times of the peaks for bilobalide, ginkgolide A, ginkgolide B, and ginkgolide C of the Sample solution correspond to those of the Standard solutions, as obtained in the test for Content of Terpene Lactones.

**Notes:**
- 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.**

**System suitability requirements**
- **Relative standard deviation:** NMT 2.0% determined from the quercetin peak in repeated injections, **Standard solution A**
- **Suitability requirements**
  - **Relative standard deviation:** NMT 2.0% determined from the quercetin peak in repeated injections, **Standard solution A**
  - **Sample solution:** Weigh and finely powder the contents of NLT 20 Capsules. Transfer an accurately weighed quantity of the powder, equivalent to about 120 mg of Powdered Ginkgo Extract, to a 30-mL glass centrifuge tube with a screw cap and PTFE gasket. Add 10.0 mL of Solvent, seal the tube, and mix well on a vortex mixer. Heat in a water bath at 90° for 30 min. Mix the hot suspension on a vortex mixer, and repeat the heating at...
90° for 30 min. Cool, centrifuge, transfer the supernatant to a flask, and return the residue to the glass tube. Repeat the extraction two more times, each time using 10.0 mL of Solvent. Combine the extracts, allow them to cool to room temperature, and evaporate to dryness under vacuum in a water bath maintained at 50°. 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. [NOTE—Do not exceed 20 mL of total aqueous phase or the holding capacity of the chromatographic tube.] Allow the Buffer solution to be absorbed into the column. After 15 min, elute the column with 100 mL of ethyl acetate, collect the ethyl acetate solution, and evaporate to dryness under vacuum in a water bath maintained at 50°. Dissolve the residue in 20.0 mL of Diluent.

Chromatographic system
(See Chromatography [621], System Suitability.)
Mode: LC
Detector: Evaporative light-scattering. [NOTE—The parameters of the detector are adjusted to achieve the best signal-to-noise ratio, according to manufacturer recommendations.]
Column: 4.6-mm × 25-cm; packing L1
Column temperature: 25 ± 1°
Flow rate: 1 mL/min
Injection volume: 15 μL
System suitability
Samples: Standard solutions
Suitability requirements:
Chromatogram similarity: The chromatograms from the Standard solutions are similar to the reference chromatogram provided with the lot of USP Ginkgo Terpene Lactones RS being used.
Relative standard deviation: NMT 2.0% determined from the bilobalide peak in repeated injections
Correlation coefficient: NLT 0.995 for the regression line as determined in Analysis

Analysis
Samples: Standard solutions and Sample solution
Record the chromatograms, and identify the peaks of the relevant analytes in the chromatogram of the Standard solutions by comparison with the reference chromatogram of the USP Ginkgo Terpene Lactones RS lot being used. Measure the areas of the analyte peaks. Plot the logarithms of the relevant peak responses versus the logarithms of concentrations, in mg/mL, of each analyte obtained from the Standard solutions, and determine the regression line using a least-squares analysis. From the graphs, determine the concentration, C, in mg/mL, of the relevant analyte in the Sample solution. Separately calculate the quantities, in mg, of bilobalide (C$_{20}$H$_{24}$O$_{9}$), ginkgolide A (C$_{20}$H$_{24}$O$_{10}$), ginkgolide B (C$_{20}$H$_{24}$O$_{11}$), and ginkgolide C (C$_{20}$H$_{24}$O$_{12}$) in the portion of Capsules taken:

Result = C × 20

C = concentration of the relevant analyte in the Sample solution (mg/mL)

Calculate the total quantity of terpene lactones in the portion of Capsules taken by adding the quantities calculated for each analyte. Calculate the total quantity, in mg, of terpene lactones per Capsule and the percentage of terpene lactones in the labeled amount of Powdered Ginkgo Extract.

Acceptance criteria: 5.4%–12.0% of terpene lactones, calculated as the sum of bilobalide, ginkgolide A, ginkgolide B, and ginkgolide C

Performance Tests
• Disintegration and Dissolution (2040): Meet the requirements for Dissolution
  Medium: 0.1 N hydrochloric acid; 500 mL
  Apparatus 2: 75 rpm
  Time: 45 min
  Standard solutions: Proceed as directed in the test for Content of Terpene Lactones.
  Sample solution: Combine 25-mL portions of the solution under test from each of the six dissolution vessels in a separation funnel. Extract with four 50-mL portions of ethyl acetate. Combine the extracts and evaporate in vacuum to dryness. Dissolve the residue with sonication in 5.0 mL of a mixture of water and methanol (1:1).
  Analysis: Proceed as directed in the test for Content of Terpene Lactones to determine the concentration, C, in mg/mL, of ginkgolide B in the Sample solution. Calculate the percentage of ginkgolide B dissolved:

  Result = 5000C/3G

  C = concentration of ginkgolide B in the Sample solution (mg/mL)

  G = content of ginkgolide B as determined in the test for Content of Terpene Lactones (mg/Capsule)

  Tolerances: NLT 75% of the content of ginkgolide B is dissolved.

  • Weight Variation (2091): Meet the requirements

Contaminants
Add the following:

• Microbial Enumeration Tests (2021): The total aerobic microbial count does not exceed 10$^4$ cfu/g, and the total combined molds and yeasts count does not exceed 10$^3$ cfu/g. (USP35)

Add the following:

• Absence of Specified Microorganisms (2022): Meet the requirements of the tests for absence of Salmonella species and Escherichia coli. (USP35)

Additional Requirements
• Packaging and Storage: Preserve in tight, light-resistant containers, and store at room temperature.

Labeling: The label states the Latin binomial and, following the official name, the article used to prepare the Capsules. Label the Capsules to indicate the amount, in mg, of Powdered Ginkgo Extract per Capsule.

Change to read:

• USP Reference Standards (11)
  USP Ginkgo Terpene Lactones RS
  USP Isorhamnetin RS
  USP Kaempferol RS
  USP Quercetin RS

---

1 Suitable commercially available material is Extrelut® NT 20 from E Merck Science.