Ginkgo Tablets

DEFINITION

Ginkgo Tablets are prepared from 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, consisting 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 ($C_{20}H_{24}O_{11}$).

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.▲*USP35*
- **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 NLT 20 Tablets. 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 glass vial. Heat in a steam bath for 25 min, and cool to room temperature in an ice bath.

  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 quantity, in mg, of each flavonol glycoside in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times C_S \times F \times 50$$

  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)

  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 quantity, in mg, of flavonol glycosides in the portion of Tablets taken by adding the individual quantities calculated. Calculate the total quantity, in mg, of flavonol glycosides per Tablet and the percentage of flavonol glycosides in the labeled amount of Powdered Ginkgo Extract.

  Acceptance criteria: 22.0%–27.0% of flavonol glycosides▲*USP35*

Change to read:

- **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.• (RB 1-Nov-2011)

  Sample solution: Weigh and finely powder NLT 20 Tablets. 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 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 of 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 ($C_{15}H_{18}O_8$), 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 Tablets taken:

$$\text{Result} = C \times 20$$

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

Calculate the total quantity of terpene lactones in the portion of Tablets taken by adding the quantities calculated for each analyte. Calculate the total quantity, in mg, of terpene lactones per Tablet and the percentage of terpene lactones in the labeled amount of Powdered Ginkgo Extract.
Acceptance criteria: 5.4%–12.0% of terpene lactones, consisting 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:

$$\text{Result} = 5000C/3G$$

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/Tablet)

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 Tablets. Label the Tablets to indicate the content, in mg, of Powdered Ginkgo Extract per Tablet.

Change to read:

- **USP REFERENCE STANDARDS ⟨11⟩**
USP Ginkgo Terpene Lactones RS
▲USP Isorhamnetin RS
USP Kaempferol RS▲*USP35*
USP Quercetin RS

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