Calculate the total percentage of terpene lactones in the portion of Ginkgo taken by adding the percentages calculated for each analyte.

Acceptance criteria: NLT 0.1% of terpene lactones, calculated as the sum of bilobalide, ginkgolide A, ginkgolide B, and ginkgolide C, on the dried basis

CONTAMINANTS

- **Articles of Botanical Origin,** *Pesticide Residue Analysis* **⟨561⟩:** Meets the requirements
- **Articles of Botanical Origin,** *Limits of Elemental Impurities* **⟨561⟩:** Meets the requirements
- **Microbial Enumeration Tests ⟨2021⟩:** The total aerobic bacterial count does not exceed 10^5 cfu/g, the total combined molds and yeasts count does not exceed 10^3 cfu/g, and the bile-tolerant Gram-negative bacteria do not exceed 10^3 cfu/g.
- **Absence of Specified Microorganisms ⟨2022⟩:** Meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*

SPECIFIC TESTS

- **Botanical Characteristics**

Macroscopic: Dried whole, folded, or fragmented leaves, with or without attached petiole, vary from khaki green to greenish brown in color; often more brown at the apical edge, and darker on the adaxial surface. Laminae are broadly obcuneate (fan-shaped), 2–12 cm in width and 2–9.5 cm in length from petiole to apical margin; mostly 1.5–2 times wider than long. The base margins are entire, concave; apical margin sinuate, usually truncate or centrally cleft, and rarely multiply cleft. The surface is glabrous, with wrinkled appearance due to prominent dichotomous venation appearing parallel and extending from the lamina base to the apical margin. Petioles, similar in color to leaf, are channeled on the adaxial surface, and 2–8 cm in length.

Microscopic

Transverse section of lamina: A thin but marked cuticle occurs over a single layer of epidermal cells on both surfaces. Stomata are present on the lower surface only, with guard cells sunken with respect to adjacent epidermal cells. Palisade elements, elongated, at right angles to the surface and often irregular in appearance, occur just below the upper epidermis. Vascular bundles occur at intervals along the width of the blade, with adjacent cluster crystals of calcium oxalate. Cells of the mesophyll are smaller than the palisade cells, elongated, parallel to the leaf surface, and separated by large intercellular spaces.

Powdered lamina and petiole: Under the microscope, transverse fragments of the leaf display a smooth cuticle, present on both leaf surfaces and staining pinkish orange with sudan III TS. In surface view, cells of the upper epidermis are elongated and wavy-walled, with abundant yellow droplets 2–12 µm in diameter visible in mature and old leaves but not in young leaves. Cells of the lower epidermis are similar in shape but have straighter walls and are interrupted by anisocytic stomata. Numerous lignified elements derived from the lamina and petiole are present, including xylem vessels with annular thickening, tracheids, and vessels with bordered pits. The extent of lignification, particularly in the petiole, increases with age of leaf. Calcium oxalate crystals are numerous, scattered or associated with vessels, ranging in size from 5 to 50 µm in young leaves and 15 to 100 µm in mature leaves. Under crossed polarizers, numerous smaller prism- or tear-shaped shiny features of indeterminate nature may be present. Very occasional, highly elongated, uniseriate, covering trichomes with no obvious cross walls and smooth or warty surfaces may be seen. Mature leaves may show the presence of very rare, polygonal to circular starch granules approximately 20 µm in diameter, with a central hilum and exhibiting a marked Maltese cross under crossed polarizers.

- **Articles of Botanical Origin,** *Foreign Organic Matter* **⟨561⟩:** NMT 3.0% of stems and NMT 2.0% of other foreign organic matter
- **Loss on Drying ⟨731⟩**

Sample: 1.0 g of finely powdered Ginkgo
Analysis: Dry the *Sample* at 105° for 2 h.
Acceptance criteria: NMT 11.0%

- **Articles of Botanical Origin,** *Total Ash* **⟨561⟩**

Sample: 1.0 g of finely powdered Ginkgo
Acceptance criteria: NMT 11.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers, protected from light and moisture, and store at room temperature.
- **Labeling:** The label states the Latin binomial and, following the official name, the part of the plant contained in the article.
- **USP Reference Standards ⟨11⟩**

USP Chlorogenic Acid RS
USP Ginkgo Terpene Lactones RS
USP Isorhamnetin RS
USP Kaempferol RS
USP Quercetin RS
USP Rutin RS

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

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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* 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*.)

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

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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 chromatograms of the *Standard solutions*, by comparing them 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 by 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 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:

$$\text{Result} = (C/W) \times 2000$$

C = concentration of the relevant analyte in the *Sample solution* (mg/mL)
W = weight of Powdered Extract taken to prepare the *Sample solution* (mg)

Calculate the total percentage of terpene lactones in the portion of Powdered Extract taken by adding the percentages calculated for each analyte.

Acceptance criteria
Total terpene lactones: 5.4%–12.0%
Bilobalide: 2.6%–5.8%
Sum of ginkgolide A, ginkgolide B, and ginkgolide C: 2.8%–6.2%

CONTAMINANTS

- **ARTICLES OF BOTANICAL ORIGIN,** *Pesticide Residues* ⟨561⟩: Meets the requirements

Delete the following:

- •**HEAVY METALS,** *Method II* ⟨231⟩: NMT 20 µg/g• (Official 1-Jan-2018)
- **MICROBIAL ENUMERATION TESTS** ⟨2021⟩: The total aerobic bacterial count does not exceed 10^4 cfu/g, and the total combined molds and yeasts count does not exceed 10^3 cfu/g.
- **ABSENCE OF SPECIFIED MICROORGANISMS** ⟨2022⟩: Meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli*

SPECIFIC TESTS

- **LIMIT OF RUTIN AND QUERCETIN**

Solution A: 0.1% of formic acid in water
Solution B: Acetonitrile
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	90	10
40	64	36
45	0	100
50	0	100
51	90	10
60	90	10

Standard solution: Prepare a composite solution of 0.4 mg/mL USP Rutin RS and 0.05 mg/mL USP Quercetin RS in methanol. Sonicate to dissolve, if necessary, and mix well.
Sample solution: Transfer 100 mg of Powdered Extract into a 10-mL volumetric flask. Add about 7 mL of methanol, and sonicate to dissolve. Dilute with methanol to volume, and mix well. Pass through a filter of 0.45-µm or finer pore size.

Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; 5-µm base-deactivated packing L1
Column temperature: 30°
Flow rate: 1.0 mL/min
Injection volume: 10 µL

System suitability
Sample: *Standard solution*
[NOTE—The relative retention times are 1.0 and 1.8 for rutin and quercetin, respectively.]
Suitability requirements
Column efficiency: NLT 15,000 theoretical plates for the rutin peak and NLT 20,000 for the quercetin peak
Tailing factor: 0.8–2.0 for the rutin peak
Relative standard deviation: NMT 2.0% for the rutin peak in repeated injections
[NOTE—If deterioration of peak shapes is observed, wash the column using a mixture of acetonitrile and water (9:1) at 1.0 mL/min for 30 min.]

Analysis
Samples: *Standard solution* and *Sample solution*
Use the chromatogram of the *Standard solution* to identify the rutin and quercetin peaks.
Calculate the percentages of rutin and quercetin in the portion of Powdered Extract taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of the relevant analyte from the *Sample solution*
r_S = peak area of the relevant analyte from the *Standard solution*
C_S = concentration of USP Rutin RS or USP Quercetin RS in the *Standard solution* (mg/mL)
C_U = concentration of Powdered Extract in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 4% of rutin and NMT 0.5% of quercetin

- **LIMIT OF GINKGOLIC ACIDS**

Solution A: 0.01% phosphoric acid in water
Solution B: 0.01% phosphoric acid in acetonitrile
Mobile phase: See *Table 3*.

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Table 3

Time (min)	Solution A (%)	Solution B (%)
0	25	75
6	10	90
7	10	90
8	25	75
10	25	75

Standard solution: Dissolve USP Ginkgolic Acids RS in methanol, and dilute, if necessary, with water to obtain a concentration of 0.25 µg/mL of ginkgolic acids, calculated as the sum of the congeners ginkgolic acid C13:0, ginkgolic acid C15:1, and ginkgolic acid C17:1.
Sample solution: Transfer 0.5 g of Powdered Extract to a 10-mL volumetric flask. Add 8 mL of methanol to dissolve, and dilute with water to volume.
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 5-cm; base-deactivated packing L7
Column temperature: 35°
Flow rate: 1 mL/min
Injection volume: 100 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Chromatogram similarity: The chromatogram is similar to the reference chromatogram provided with the lot of USP Ginkgolic Acids RS being used.
Tailing factor: NMT 2.0 for the ginkgolic acid C15:1 peak
Relative standard deviation: NMT 5.0% for the ginkgolic acid C15:1 peak in repeated injections
Analysis
Samples: *Standard solution* and *Sample solution*
[NOTE—Identify the peaks of the relevant analytes by comparison with the reference chromatogram of the USP Ginkgolic Acids RS lot being used. If deterioration of peak shapes is observed, wash the column using a mixture of methanol and water (9:1) for 30 min.]
Calculate the concentration, in µg/g, of each ginkgolic acid in the portion of Powdered Extract taken:

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

r_U = peak area of the relevant analyte from the *Sample solution*
r_S = peak area of the relevant analyte from the *Standard solution*
C_S = concentration of USP Ginkgolic Acids RS in the *Standard solution* (mg/mL)
W = weight of Powdered Extract taken to prepare the *Sample solution* (mg)
P = content of the relevant ginkgolic acid in USP Ginkgolic Acids RS (µg/g)

Calculate the total amount of ginkgolic acids by adding the individual contents.
Acceptance criteria: NMT 5 µg/g

- **LOSS ON DRYING** ⟨731⟩
  Sample: 1.0 g of Powdered Extract
  Analysis: Dry the *Sample* at 105° for 2 h.
  Acceptance criteria: NMT 5.0%
- **OTHER REQUIREMENTS:** Meets the requirements for *Residual Solvents* in *Botanical Extracts* ⟨565⟩

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, protect 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 Isorhamnetin RS
  USP Kaempferol RS
  USP Quercetin RS
  USP Rutin RS

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 ($C_{15}H_{18}O_8$), 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

- **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

- **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 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

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