COMPOSITION

• **CONTENT OF GYMNEMIC ACIDS**

Solution A: Dissolve 0.14 g of anhydrous potassium dihydrogen phosphate in 900 mL of water, and add 0.5 mL of phosphoric acid. Dilute with water to 1000 mL, mix, filter, and degas.

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	75	25
20	45	55
25	40	60
30	40	60
35	75	25
40	75	25

Solvent: 50% ethanol in water

Potassium hydroxide solution: 12% potassium hydroxide in water

Hydrochloric acid solution: 4 N hydrochloric acid

Standard solution A: 0.3 mg/mL of USP Gymnemagenin RS in methanol

Standard solution B: Transfer about 0.25 g of USP Native Gymnema Extract RS to a 100-mL round-bottom flask fitted with a reflux condenser. Add 25 mL of *Solvent* and 2 mL of *Potassium hydroxide solution*, reflux on a water bath for 1 h, and cool to room temperature. Add 5.5 mL of *Hydrochloric acid solution*, reflux on a water bath for 2 h, and cool to room temperature. Adjust the solution with *Potassium hydroxide solution* to a pH of 7.5–8.5, transfer to a 50-mL volumetric flask, dilute with *Solvent* to volume, and mix. Before injection, pass through a membrane filter having a 0.45-µm or finer pore size, discarding the first few mL of the filtrate.

Sample solution: Transfer an amount of Native Gymnema Extract, equivalent to about 30 mg of gymnemic acids, to a 100-mL round-bottom flask fitted with a reflux condenser. Add 25 mL of *Solvent* and 2 mL of *Potassium hydroxide solution*, reflux on a water bath for 1 h, and cool to room temperature. Add 5.5 mL of *Hydrochloric acid solution*, reflux on a water bath for 2 h, and cool to room temperature. Adjust the solution with *Potassium hydroxide solution* to a pH of 7.5–8.5, transfer to a 100-mL volumetric flask, dilute with *Solvent* to volume, and mix. Before injection, pass through a membrane filter having a 0.45-µm or finer pore size, discarding the first few mL of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; 5-µm, 100 Å, packing L1

Column temperature: 25 ± 1°

Flow rate: 1.6 mL/min

Injection volume: 20 µL

System suitability

Samples: *Standard solution A* and *Standard solution B*

Suitability requirements

Chromatogram similarity: The chromatogram from *Standard solution B* is similar to the reference chromatogram provided with the lot of USP Native Gymnema Extract RS being used.

Tailing factor: NMT 1.5 for the gymnemagenin peak, *Standard solution A*

Relative standard deviation: NMT 2.0% determined from the gymnemagenin peak in repeated injections, *Standard solution A*

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Using the chromatograms of *Standard solution A*, *Standard solution B*, and the reference chromatogram provided with the lot of USP Native Gymnema Extract RS being used, identify the retention times of the peaks corresponding to deacylgymnemic acid and gymnemagenin from the *Sample solution*.

Calculate the percentage of gymnemic acids, calculated as gymnemagenin, in the portion of Native Gymnema Extract taken:

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

r_U = peak area of gymnemagenin from the *Sample solution*

r_S = peak area of gymnemagenin from *Standard solution A*

C_S = concentration of gymnemagenin in *Standard solution A* (mg/mL)

C_U = concentration of Native Gymnema Extract in the *Sample solution* (mg/mL)

Acceptance criteria: NLT 5.0% on the dried basis

CONTAMINANTS

Delete the following:

•• **HEAVY METALS,** *Method III* ⟨**231**⟩**:** NMT 20 µg/g •• (Official 1-Jan-2018)

• **ARTICLES OF BOTANICAL ORIGIN,** *General Method for Pesticide Residues Analysis* ⟨**561**⟩**:** Meets the requirements

• **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 absence of *Salmonella* species and *Escherichia coli*

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨**731**⟩

Sample: 1.0 g of Native Gymnema Extract

Analysis: Dry at 105° for 3 h.

Acceptance criteria: NMT 5.0%

• **ARTICLES OF BOTANICAL ORIGIN,** *Total Ash* ⟨**561**⟩

Sample: 1.0 g of Native Gymnema Extract

Acceptance criteria: NMT 8%

• **ARTICLES OF BOTANICAL ORIGIN,** *Acid-Insoluble Ash* ⟨**561**⟩

Sample: 1.0 g of Native Gymnema Extract

Acceptance criteria: NMT 2.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light and moisture. 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 derived. It meets other labeling requirements under *Botanical Extracts* ⟨565⟩.

• **USP REFERENCE STANDARDS** ⟨**11**⟩

USP Gymnemagenin RS

USP Native Gymnema Extract RS

Purified Gymnema Extract

DEFINITION

Purified Gymnema Extract is prepared from Native Gymnema Extract by precipitation using dilute hydrochloric acid solution. The ratio of plant material to extract is about 25:1. It contains NLT 90.0% and NMT 110.0% of

DS Monographs

the labeled amount of gymnemic acids, calculated as gymnemagenin on the dried basis. It may contain suitable added substances as carriers.

IDENTIFICATION

• **A. Thin-Layer Chromatography**

Standard solution A: 0.5 mg/mL of USP Gymnemagenin RS in methanol

Standard solution B: 20 mg/mL of USP Native Gymnema Extract RS in methanol. Sonicate for 10 min, centrifuge, and use the supernatant.

Sample solution: 20 mg/mL of Purified Gymnema Extract in methanol. Sonicate for 10 min, centrifuge, and use the supernatant.

Chromatographic system

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

Application volume: 5 µL as 8-mm bands

Relative humidity: Condition the plate to a relative humidity of about 33%, using a suitable device.

Developing solvent system: A mixture of dichloromethane, methanol, and formic acid (75:25:10)

Developing distance: 6 cm

Derivatization reagent: A mixture of methanol and sulfuric acid (9:1)

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*. Apply the samples as bands to a suitable high-performance thin-layer chromatographic plate, and dry in air (see *Chromatography* ⟨621⟩). Develop the chromatograms in a saturated chamber, remove the plate from the chamber, dry in air, derivatize the plate with *Derivatization reagent*, heat at 110° for 3 min, and examine under visible light and UV at 366 nm.

System suitability: The chromatogram of *Standard solution B* shows two bands clearly separated at an R_F of about 0.6–0.7 below the band due to gymnemagenin in *Standard solution A*, and the most prominent band is located at about one-third of the chromatogram, visible as brown in color under white light and blue under UV.

Acceptance criteria: The chromatogram of the *Sample solution* shows the following bands corresponding in color and position to bands in the chromatogram of *Standard solution B*: two bands at an R_F of about 0.6–0.7, below the band due to gymnemagenin in *Standard solution A*; the most prominent band at about one-third of the chromatogram, visible as brown in color under white light and blue under UV; and in the lower third of the chromatogram, under UV, one light blue-greenish band and a dark band underneath.

• **B. HPLC**

Analysis: Proceed as directed in the test for *Content of Gymnemic Acids*.

Acceptance criteria: The chromatogram of the *Sample solution* shows a major peak at a retention time corresponding to that of the gymnemagenin peak in the chromatogram of *Standard solution A* and an additional peak corresponding to deacylgymnemic acid.

COMPOSITION

• **Content of Gymnemic Acids**

Solution A: Dissolve 0.14 g of anhydrous potassium dihydrogen phosphate in 900 mL of water, and add 0.5 mL of phosphoric acid. Dilute with water to 1000 mL, mix, filter, and degas.

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	75	25
20	45	55
25	40	60
30	40	60
35	75	25
40	75	25

Solvent: 50% ethanol in water

Potassium hydroxide solution: 12% potassium hydroxide in water

Hydrochloric acid solution: 4 N hydrochloric acid

Standard solution A: 0.3 mg/mL of USP Gymnemagenin RS in methanol

Standard solution B: Transfer about 0.25 g of USP Native Gymnema Extract RS to a 100-mL round-bottom flask fitted with a reflux condenser. Add 25 mL of *Solvent* and 2 mL of *Potassium hydroxide solution*, reflux on a water bath for 1 h, and cool to room temperature. Add 5.5 mL of *Hydrochloric acid solution*, reflux on a water bath for 2 h, and cool to room temperature. Adjust the solution with *Potassium hydroxide solution* to a pH of 7.5–8.5, transfer to a 50-mL volumetric flask, dilute with *Solvent* to volume, and mix. Before injection, pass through a membrane filter having a 0.45-µm or finer pore size, discarding the first few mL of the filtrate.

Sample solution: Transfer an amount of Purified Gymnema Extract, equivalent to about 30 mg of gymnemic acids, to a 100-mL round-bottom flask fitted with a reflux condenser. Add 25 mL of *Solvent* and 2 mL of *Potassium hydroxide solution*, reflux on a water bath for 1 h, and cool to room temperature. Add 5.5 mL of *Hydrochloric acid solution*, reflux on a water bath for 2 h, and cool to room temperature. Adjust the solution with *Potassium hydroxide solution* to a pH of 7.5–8.5, transfer to a 100-mL volumetric flask, dilute with *Solvent* to volume, and mix. Before injection, pass through a membrane filter having a 0.45-µm or finer pore size, discarding the first few mL of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; 5-µm, 100 Å, packing L1

Column temperature: 25 ± 1°

Flow rate: 1.6 mL/min

Injection volume: 20 µL

System suitability

Samples: *Standard solution A* and *Standard solution B*

Suitability requirements

Chromatogram similarity: The chromatogram from *Standard solution B* is similar to the reference chromatogram provided with the lot of USP Native Gymnema Extract RS being used.

Tailing factor: NMT 1.5 for the gymnemagenin peak, *Standard solution A*

Relative standard deviation: NMT 2.0% for the gymnemagenin peak, *Standard solution A*

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Using the chromatograms of *Standard solution A*, *Standard solution B*, and the reference chromatogram provided with the lot of USP Native Gymnema Extract RS being used, identify the retention times of the peaks corresponding to deacylgymnemic acid and gymnemagenin in the *Sample solution* chromatogram.

Calculate the percentage (P) of gymnemic acids, calculated as gymnemagenin, in the portion of Purified Gymnema Extract taken:

$$P = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of gymnemagenin from the *Sample solution*
r_S = peak area of gymnemagenin from *Standard solution A*
C_S = concentration of gymnemagenin in *Standard solution A* (mg/mL)
C_U = concentration of Purified Gymnema Extract in the *Sample solution* (mg/mL)

Calculate the percentage of the labeled amount of gymnemic acids in the portion of Purified Gymnema Extract taken:

$$\text{Result} = (P/L) \times 100$$

P = content of gymnemic acids as determined above (%)
L = labeled amount of gymnemic acids (%)

Acceptance criteria: 90.0%–110.0% of the labeled amount on the dried basis

CONTAMINANTS

Delete the following:

- **HEAVY METALS,** *Method III* ⟨**231**⟩**:** NMT 20 µg/g● (Official 1-Jan-2018)
- **ARTICLES OF BOTANICAL ORIGIN,** *General Method for Pesticide Residues Analysis* ⟨**561**⟩**:** Meets the requirements
- **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 absence of *Salmonella* species and *Escherichia coli*

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨**731**⟩
  Sample: 1.0 g of Purified Gymnema Extract
  Analysis: Dry at 105° for 3 h.
  Acceptance criteria: NMT 5.0%
- **ARTICLES OF BOTANICAL ORIGIN,** *Total Ash* ⟨**561**⟩
  Sample: 1.0 g of Purified Gymnema Extract
  Acceptance criteria: NMT 8%
- **ARTICLES OF BOTANICAL ORIGIN,** *Acid-Insoluble Ash* ⟨**561**⟩
  Sample: 1.0 g of Purified Gymnema Extract
  Acceptance criteria: NMT 2.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light and moisture. 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 derived. Label it to indicate the content of gymnemic acids, in percentage. It meets other labeling requirements under *Botanical Extracts* ⟨565⟩.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Gymnemagenin RS
  USP Native Gymnema Extract RS

Hawthorn Leaf with Flower

DEFINITION

Hawthorn Leaf with Flower consists of the dried tips of the flower-bearing branches up to 7 cm in length of *Crataegus monogyna* Jacq. emend Lindman. or *Crataegus laevigata* (Poir.) DC., also known as *Crataegus oxycantha* Linné (Fam. Rosaceae). It contains NLT 0.6% of *C*-glycosylated flavones, expressed as vitexin ($C_{21}H_{20}O_{10}$), and NLT 0.45% of *O*-glycosylated flavones, expressed as hyperoside ($C_{21}H_{20}O_{12}$), calculated on the dried basis.

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨**201**⟩
  Standard solution: 0.1 mg/mL each of chlorogenic acid, rutin, USP Hyperoside RS, and USP Vitexin RS in methanol. [NOTE—Reserve a portion of this solution for use in *Identification* test *B*.]
  Sample solution: Finely powder 10 g of Hawthorn Leaf with Flower. Transfer 1 g of the powder to a flask, and add 10 mL of methanol. Heat the flask on a water bath maintained at 65° for 5 min, cool, filter, and use the filtrate.
  Adsorbent: 0.50-mm layer of chromatographic silica gel mixture (TLC plates)
  Application volume: 10 µL
  Developing solvent system: Ethyl acetate, glacial acetic acid, formic acid, and water (10: 1.1: 1.1: 2.6)
  Derivatization reagent A: 2-Aminoethyl diphenylborinate in methanol (1 in 100)
  Derivatization reagent B: Polyethylene glycol 4000 in methanol (5 in 100)
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Proceed as directed in the chapter, except to dry the plate at 105°, and spray the plate while still hot with 10 mL of *Derivatization reagent A* and then with 10 mL of *Derivatization reagent B*. Allow the plate to air-dry for 30 min, and examine under long-wave UV light (365 nm).
  Acceptance criteria: The chromatogram of the *Standard solution* exhibits an intense orange zone (at R_F value of 0.3) due to rutin; a light blue fluorescent zone (at R_F value of 0.4) due to chlorogenic acid; a yellowish-orange zone (at R_F value of 0.55) due to hyperoside; and a yellowish-green zone (at R_F value of 0.65) due to vitexin. The chromatogram of the *Sample solution*, in addition to the zones due to rutin, chlorogenic acid, hyperoside, and vitexin, exhibits a yellowish-green zone (at R_F value of 0.35) due to vitexin-2-rhamnoside; a light blue fluorescent zone (at R_F value of 0.6) due to spiraeoside; and a light blue fluorescent zone near the solvent front (at R_F value of 0.9) due to caffeic acid. The chromatogram of the *Sample solution* also exhibits additional zones of weaker intensity.
- **B. HPLC**
  Solution A: Tetrahydrofuran, acetonitrile, and methanol (92.4: 3.4: 4.2)
  Solution B: 0.5% Phosphoric acid in water
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	12	88
12	12	88
25	18	82
30	18	82

Standard solution: Use the *Standard solution* reserved from *Identification* test *A*.
Sample solution: Transfer 3 g of finely powdered Hawthorn Leaf with Flower to a 100-mL round-bottom flask, add 60 mL of a mixture of methanol and water (4:1), and maintain under reflux for 1 h. Cool, filter, and collect the filtrate in a separate flask. Transfer the residue from the filter back to the flask, add 40 mL of a mixture of methanol and water (4:1), and maintain under reflux

DS Monographs