• **USP REFERENCE STANDARDS ⟨11⟩**
USP *Rhodiola rosea* Root and Rhizome Dry Extract RS
USP Rosavin RS
USP Salidroside RS

Powdered *Rhodiola rosea* Extract

DEFINITION

Powdered *Rhodiola rosea* Extract is prepared from *Rhodiola rosea* by extraction with hydroalcoholic mixtures. The ratio of plant material to extract is between 1.5: 1 and 5:1. It contains NLT 90.0% and NMT 110.0% of the labeled amount of phenylpropenoid glycosides calculated as the sum of rosarin, rosavin, and rosin, and NLT 90.0% and NMT 110.0% of the labeled amount of salidroside, both calculated on the dried basis. It may contain suitable added substances as carriers.

IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHY**

Standard solution A: 1.0 mg/mL of USP Rosavin RS in methanol

Standard solution B: 50 mg/mL of USP *Rhodiola rosea* Root and Rhizome Dry Extract RS in methanol. Sonicate for 10 min, centrifuge, and use the supernatant.

Sample solution: 50 mg/mL of Powdered *Rhodiola rosea* Extract in methanol. Sonicate for 10 min, centrifuge, and use the supernatant.

Chromatographic system
(See *Chromatography ⟨621⟩, Thin-Layer Chromatography*.)

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

Application volume: 3 µL of *Standard solution A* and 5 µL each of *Standard solution B* and the *Sample solution*; 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 ethyl acetate, methanol, water, and formic acid (77:13:10:2)

Developing distance: 6 cm

Derivatization reagent: Dissolve 1 g of diphenylamine in 40 mL of acetone, add 1 mL of aniline, and mix. Carefully add 7.5 mL of phosphoric acid, and mix.

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. Develop the chromatograms in a saturated chamber, remove the plate from the chamber, and dry in air. Derivatize the plate with *Derivatization reagent*, heat at 120° for 5 min, and examine under visible light.

System suitability: The chromatogram of *Standard solution B* exhibits, in the lower half, three gray bands and two brownish bands, one above and the other below the gray bands; the most intense band in the chromatogram is the brownish band with an R_F below the gray bands; the most intense gray band is the lower band at an R_F corresponding to the band due to rosavin in the chromatogram of *Standard solution A*; the upper gray band due to rosarin is less intense.

Acceptance criteria: The chromatogram of the *Sample solution* exhibits a gray band corresponding to the band due to rosavin in the chromatogram of *Standard solution A*, and the following bands corresponding to similar bands in the chromatogram of *Standard solution B*: two additional gray bands and two brownish bands, one above and the other below the gray bands; the most intense band in the chromatogram is the brownish band with an R_F below the gray bands; the most intense gray band is the lower band due to rosavin.

• **B. HPLC**

Analysis: Proceed as directed in the test for *Content of Phenylpropenoid Glycosides and Salidroside*.

Acceptance criteria: The chromatogram of the *Sample solution* exhibits peaks at the retention times corresponding to the peaks due to salidroside, tyrosol, rosarin, rosavin, rosin, and rosiridin in the chromatogram of *Standard solution C*. The ratio of the contents of rosarin, rosavin, and rosin is about 2.5: 6.0: 1.5, respectively.

COMPOSITION

• **CONTENT OF PHENYLPROPENOID GLYCOSIDES AND SALIDROSIDE**

Solution A: Water

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	94	6
6	83	17
7	80.3	19.7
9	80.3	19.7
10	0	100
12	94	6
17	94	6

Standard solution A: 1.0 mg/mL of USP Rosavin RS in methanol

Standard solution B: 0.3 mg/mL of USP Salidroside RS in methanol

Standard solution C: 4.0 mg/mL of USP *Rhodiola rosea* Root and Rhizome Dry Extract RS in methanol. Sonicate to dissolve, if necessary. Before injection, pass through a membrane filter of 0.45-µm or finer pore size.

Sample solution: 4.0 mg/mL of Powdered *Rhodiola rosea* Extract in methanol. Sonicate to dissolve, if necessary. Before injection, pass through a membrane filter of 0.45-µm or finer pore size.

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

Mode: LC

Detector: UV 205 nm

Column: 3.0-mm × 10-cm; 2.5-µm packing L1

Column temperature: 40 ± 1°

Flow rate: 1.0 mL/min

Injection volume: 1 µL

System suitability

Samples: *Standard solution A* and *Standard solution C*

Suitability requirements

Chromatogram similarity: The chromatogram obtained from *Standard solution C* is similar to the reference chromatogram provided with the lot of USP *Rhodiola rosea* Root and Rhizome Dry Extract RS being used.

Resolution: NLT 1.5 between the rosarin and rosavin peaks, *Standard solution C*

Relative standard deviation: NMT 2% determined from the rosavin peak in repeated injections, *Standard solution A*

Analysis

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

Using the chromatograms of *Standard solution A, Standard solution B, Standard solution C,* and the reference chromatogram provided with the lot of USP *Rhodiola rosea* Root and Rhizome Dry Extract RS being used, identify the retention time of the peaks corresponding to salidroside, tyrosol, rosarin, rosavin, rosin, and rosiridin from the *Sample solution*.

DS Monographs

Separately calculate the percentage of rosarin, rosavin, and rosin as rosavin in the portion of Powdered *Rhodiola rosea* Extract taken:

$$P_1 = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of the relevant analyte from the *Sample solution*
r_S = peak area of rosavin from *Standard solution A*
C_S = concentration of rosavin in *Standard solution A* (mg/mL)
C_U = concentration of Powdered *Rhodiola rosea* Extract in the *Sample solution* (mg/mL)

Calculate the percentage of phenylpropenoid glycosides as the sum of the percentages of rosarin, rosavin, and rosin.

Calculate the percentage of salidroside in the portion of Powdered *Rhodiola rosea* Extract taken:

$$P_2 = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of salidroside from the *Sample solution*
r_S = peak area of salidroside from *Standard solution B*
C_S = concentration of salidroside in *Standard solution B* (mg/mL)
C_U = concentration of Powdered *Rhodiola rosea* Extract in the *Sample solution* (mg/mL)

Calculate the percentage of the labeled amount of phenylpropenoid glycosides in the portion of Powdered *Rhodiola rosea* Extract taken:

$$\text{Result} = (P_1/L) \times 100$$

P_1 = content of phenylpropenoid glycosides, as determined above (%)
L = labeled amount of phenylpropenoid glycosides (%)

Calculate the percentage of the labeled amount of salidroside in the portion of Powdered *Rhodiola rosea* Extract taken:

$$\text{Result} = (P_2/L) \times 100$$

P_2 = content of salidroside, as determined above (%)
L = labeled amount of salidroside (%)

Acceptance criteria: NLT 90.0% and NMT 110.0% of the labeled amount of phenylpropenoid glycosides, and NLT 90.0% and NMT 110.0% of the labeled amount of salidroside, both calculated on the dried basis.

CONTAMINANTS

- **Elemental Impurities—Procedures ⟨233⟩**
  Acceptance criteria
  Arsenic: NMT 2.0 µg/g
  Cadmium: NMT 1.0 µg/g
  Lead: NMT 5.0 µg/g
  Mercury: NMT 1.0 µg/g
- **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*
- **Articles of Botanical Origin,** *Test for Aflatoxins* **⟨561⟩:** Meets the requirements

SPECIFIC TESTS

- **Loss on Drying ⟨731⟩**
  Sample: 2.0 g of Powdered *Rhodiola rosea* Extract
  Analysis: Dry at 105° for 2 h.
  Acceptance criteria: NMT 5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers, protected from light and 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 derived. It meets other labeling requirements under *Botanical Extracts* ⟨565⟩.
- **USP Reference Standards ⟨11⟩**
  USP *Rhodiola rosea* Root and Rhizome Dry Extract RS
  USP Rosavin RS
  USP Salidroside RS

Rhodiola rosea Tincture

DEFINITION

Rhodiola rosea Tincture is prepared as follows.

Rhodiola rosea	1 part (g)
A mixture of Alcohol and Water (40:60), a sufficient quantity to make	5 parts (mL)

Prepare the Tincture as directed for *Botanical Extracts* ⟨565⟩, *Tinctures, Maceration Process*. It contains NLT 0.06% (w/v) of phenylpropenoid glycosides calculated as the sum of rosarin, rosavin, and rosin; and NLT 0.016% of salidroside.

IDENTIFICATION

- **A. Thin-Layer Chromatography**
  (See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
  Standard solution A: 1.0 mg/mL of USP Rosavin RS in methanol
  Standard solution B: 50 mg/mL of USP *Rhodiola rosea* Root and Rhizome Dry Extract RS in methanol. Sonicate for 10 min, centrifuge, and use the supernatant.
  Sample solution: Centrifuge a portion of Tincture, and use the supernatant.
  Chromatographic system
  Adsorbent: Chromatographic silica gel with an average particle size of 5 µm (HPTLC plates)
  Application volume: 3 µL of *Standard solution A*, 5 µL of *Standard solution B*, and 10 µL of *Sample solution*; 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 ethyl acetate, methanol, water, and formic acid (77:13:10:2)
  Developing distance: 6 cm
  Derivatization reagent: Dissolve 1 g of diphenylamine in 40 mL of acetone, add 1 mL of aniline, and mix. Carefully add 7.5 mL of phosphoric acid, and mix.
  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. Develop the chromatograms in a saturated chamber, remove the plate from the chamber, and dry in air. Derivatize the plate with *Derivatization reagent*, heat at 120° for 5 min, and examine under visible light.
  System suitability: The chromatogram of *Standard solution B* exhibits, in the lower half, three gray bands and two brownish bands, one above and the other below

DS Monographs