**Rhodiola rosea Root and Rhizome Dry Extract**

**Final Authorized Version 1.0**

*Rhodiola rosea* Root and Rhizome Dry Extract

**DEFINITION**

The extract is prepared from *Rhodiola rosea* L. roots and rhizomes (Family Crassulaceae) by extraction with hydro-alcoholic mixtures. It may contain suitable added substances as carriers. It contains NLT 90.0% and NMT 110.0% of the labeled amount of the phenylpropenoid glycosides rosarin, *rosavin* [1], and rosin calculated as rosavin, and NLT 90.0% and NMT 110.0% of the labeled amount of *salidroside* [2], calculated on the dried basis.

**POTENTIAL CONFOUNGING MATERIALS**

Related *Rhodiola* species including *R. kirilowii*, *R. yunnanensis*, *R. crenulata*, *R. sacra*, and *R. sachalinensis*.

**CONSTITUENTS OF INTEREST**

- Phenylpropenoid glycosides: Rosarin, *rosavin* [1], rosin
- Phenylethanoids: *Salidroside* [2], tyrosol
- Monoterpene glycoside: Rosiridin

**IDENTIFICATION**

- **A. THIN-LAYER CHROMATOGRAPHY**

  **Standard solution A:** 1.0 mg/mL of USP *Rosavin* [1] RS in methanol

  **Standard solution B:** 50 mg/mL of USP *Rhodiola rosea* Root and Rhizome Dry Extract RS [3] in methanol. Sonicate for 10 min, centrifuge, and use the supernatant.

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

  **Chromatographic system**

  (See Chromatography <621> [4], 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 *Sample solution*, as 8-mm bands

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

  **Temperature:** 25°

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

**System suitability:** *Standard solution A* shows a gray band at about one-fourth of the chromatogram. The chromatogram of *Standard solution B* exhibits, in the lower half, three gray bands, the most intense gray band at an \( R_f \) corresponding to the band due to rosavin \([1]\) in the chromatogram of *Standard solution A*; the other two are above the band corresponding to rosavin \([1]\). The chromatogram of *Standard solution B* exhibits the most intense band as a brownish band with an \( R_f \) below rosavin.

**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*. It exhibits the following bands corresponding to similar bands in the chromatogram of *Standard solution B*: two additional gray bands and two brownish bands, one above the group of gray bands; the most intense band in the chromatogram is the brownish band with an \( R_f \) below the rosavin; the most intense gray band is the lower band due to rosavin.

• **B. HPLC**

**Analysis:** Proceed as directed in the *Assay 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 B*. The ratio of the contents of rosarin, rosavin, and rosin is about 2.5: 6.0: 1.5.

**ASSAY**

• **Content of Phenylpropenoid Glycosides and Salidroside**

**Solution A:** Water

**Solution B:** Acetonitrile

**Mobile phase:** See *Table 1*.

**Table 1**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Solution A (%)</th>
<th>Solution B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>94</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>83</td>
<td>17</td>
</tr>
<tr>
<td>7</td>
<td>80.3</td>
<td>19.7</td>
</tr>
<tr>
<td>9</td>
<td>80.3</td>
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</tr>
<tr>
<td>10</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>12</td>
<td>94</td>
<td>6</td>
</tr>
<tr>
<td>17</td>
<td>94</td>
<td>6</td>
</tr>
</tbody>
</table>

**Standard solution A:** 1.0 mg/mL of *USP Rosavin RS* \([1]\) in methanol

**Standard solution B:** 4.0 mg/mL of *USP Rhodiola rosea Root and Rhizome Dry Extract RS* \([3]\) in methanol. Sonicate to dissolve, if necessary. Before injection, pass through a membrane filter of 0.45-µm or finer pore size.

**Standard solution C:** 1.0 mg/mL of *USP Salidroside RS* \([2]\) in methanol
Sample solution: 4.0 mg/mL of *Rhodiola rosea* Root and Rhizome Dry Extract in methanol. Sonicate to dissolve, if necessary. Before injection, pass through a membrane filter of 0.45-µm or finer pore size, discarding the first few mL of the filtrate.

**Chromatographic system**

(See *Chromatography* <621> [4], *System Suitability.* )

Mode: LC

Detector: UV 205 nm

Column: 3.0-mm × 10-cm; 2.5-µm packing L1 (similar to Luna C18-HST)

Column temperature: 40 ± 1°

Flow rate: 1.0 mL/min

Injection volume: 1 µL

**System suitability**

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

Suitability requirements

Chromatogram similarity: The chromatogram of *Standard solution B* 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 rosarin and rosavin peaks, *Standard solution B*

Tailing factor: NMT 2.0 for the rosavin peak, *Standard solution A*

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 in the *Sample solution*.

Separately calculate the percentages of rosarin, rosavin, and rosin as rosavin:

\[ P_1 = \left( \frac{r_U}{r_S} \right) \times \left( \frac{C_S}{C_U} \right) \times 100 \]

- \( r_U \) = peak area of the relevant analyte in the *Sample solution*
- \( r_S \) = peak area of rosavin in *Standard solution A*
- \( C_S \) = concentration of rosavin in *Standard solution A* (mg/mL)
- \( C_U \) = concentration of Dry 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:

\[ P_2 = \left( \frac{r_U}{r_S} \right) \times \left( \frac{C_S}{C_U} \right) \times 100 \]

- \( r_U \) = peak area of salidroside in the *Sample solution*
- \( r_S \) = peak area of salidroside in *Standard solution C*
- \( C_S \) = concentration of salidroside in *Standard solution C* (mg/mL)
\[ C_{u} = \text{concentration of Dry Extract in the Sample solution (mg/mL)} \]

Calculate the percentage of the labeled amount of phenylpropanoid glycosides in the Dry Extract:

\[
\text{Result} = \left( \frac{P1}{L} \right) \times 100
\]

\[ P1 = \text{content of phenylpropanoid glycosides, as determined above (%)} \]
\[ L = \text{labeled amount of phenylpropanoid glycosides (%)} \]

Calculate the percentage of the labeled amount of salidroside in the Dry Extract:

\[
\text{Result} = \left( \frac{P2}{L} \right) \times 100
\]

\[ P2 = \text{content of salidroside, as determined above (%)} \]
\[ L = \text{labeled amount of salidroside (%)} \]

Acceptance criteria

**Phenylpropanoid glycosides:** 90.0%–110.0% of the labeled amount on the dried basis

**Salidroside:** 90.0%–110.0% of the labeled amount on the dried basis

CONTAMINANTS

- **Elemental Impurities—Procedures <233>** [5]
- **Acceptance criteria**
  - **Arsenic:** NMT 2.0 µg/g
  - **Cadmium:** NMT 1.0 µg/g
  - **Lead:** NMT 5.0 µg/g
  - **Mercury:** NMT 0.2 µg/g
- **Articles of Botanical Origin** [6], General Method for Pesticide Residues Analysis <561>: Meets the requirements
- **Articles of Botanical Origin** [6], Test for Aflatoxins <7> <561>: Meets the requirements
- **Microbial Enumeration Tests <61>** [8]: 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.
- **Tests for Specified Microorganisms <62>** [9]: Meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli*

SPECIFIC TESTS

- **Loss on Drying <731>** [10]
  - **Sample:** 1.0 g of Dry Extract
  - **Analysis:** Dry the Sample at 105° for 2 h.
  - **Acceptance criteria:** NMT 5.0%

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 parts of the plant from which the article was derived. It meets other labeling requirements under *Botanical Extracts* <565>. 
• **USP Reference Standards** [11]
  
  USP Aflatoxins RS [12]
  USP *Rhodiola rosea* Root and Rhizome Dry Extract RS [3]
  USP Rosavin RS [1]
  USP Salidroside RS [2]