Rhodiola rosea Root and Rhizome Powdered Extract

Proposed For Comment Version 0.2

**Rhodiola rosea Root and Rhizome Powdered Extract**

**DEFINITION**
The article is prepared from *Rhodiola rosea* L. roots and rhizomes (Family Crassulaceae) by extraction with hydro-alcoholic mixtures. The ratio of plant material to extract is between 1.5: 1 and 5:1. 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 and rosin calculated as rosavin, and NLT 90.0% and NMT 110.0% of the labeled amount of salidroside, both 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, and rosin
- Salidroside

**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* Powdered Extract RS in methanol. Sonicate for 10 min, centrifuge, and use the supernatant.
  - **Sample solution:** 50 mg/mL of Powdered 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 **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 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:** 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, below the gray bands; the most intense gray band is the lower band at an R, 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**. 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 and the other below the gray bands; the most intense band in the chromatogram is the brownish band with an R, below the gray bands; the most intense gray band is the lower band due to rosarin.

- **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>
<td>19.7</td>
</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 in methanol
Standard solution B: 4.0 mg/mL of USP Rhodiola rosea Powdered Extract RS 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 in methanol
Sample solution: 4.0 mg/mL of Powdered 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>, 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 Powdered Extract RS being used.
Resolution: NLT 1.5 between the 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 Powdered 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:

\[ P_1 = \left( \frac{r_u}{r_s} \right) \times \left( \frac{C_s}{C_u} \right) \times 100 \]

\[ r_u = \text{peak area of the relevant analyte in the Sample solution} \]
\[ r_s = \text{peak area of rosavin in Standard solution A} \]
\[ C_s = \text{concentration of rosavin in Standard solution A (mg/mL)} \]
\[ C_u = \text{concentration of Powdered Extract in the Sample solution (mg/mL)} \]
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)
- \( C_U \) = concentration of Powdered Extract in the Sample solution (mg/mL)

Calculate the percentage of the labeled amount of phenylpropenoid glycosides in the Extract:

\[ \text{Result} = \left( \frac{P_1}{L} \right) \times 100 \]

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

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

\[ \text{Result} = \left( \frac{P_2}{L} \right) \times 100 \]

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

Acceptance criteria

- Phenylpropenoid 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>**
  - 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
- **Articles of Botanical Origin, Test for Aflatoxins <561>:** Meets the requirements
- **Microbial Enumeration Tests <61>:** 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>:** Meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli*

SPECIFIC TESTS

- **Loss on Drying <731>**
  - Sample: 1.0 g of Powdered Extract
  - Analysis: Dry 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 part 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 [1]
  - USP Rhodiola rosea Powdered Extract RS
USP Rosavin RS
USP Salidroside RS