Salvia miltiorrhiza Root and Rhizome Powder

**Final Authorized Version 1.0**

*Salvia miltiorrhiza* Root and Rhizome Powder

**DEFINITION**
The article consists of the dried roots and rhizomes of *Salvia miltiorrhiza* Bunge (Family Lamiaceae) collected in spring or fall, and reduced to a powder or very fine powder. It contains NLT 0.1% of tanshinone II₅, NLT 0.2% of total tanshinones calculated as the sum of cryptotanshinone, tanshinone I, and tanshinone II₅; and NLT 3.0% of salvianolic acid B; all calculated on the dried basis.

**POTENTIAL CONFOUNDING MATERIALS**
*Salvia bowleyana* Dun
*Salvia przewalskii* Maxim.
*Salvia trijuga* Diels
*Salvia yunnanensis* C. H. Wight

**CONSTITUENTS OF INTEREST**

* Diterpenoid quinones: Tanshinone I, tashinone II₅, and cryptotanshinone
* Phenolic acids: Salvianolic acid, lithospermic acid, and rosmarinic acid

**IDENTIFICATION**

- **A. Botanic Characteristics**

  **Macroscopic:** Yellowish-brown to reddish-brown in color

  **Microscopic:** It shows fragments of cork cells, subrectangular or polygonal, containing yellowish-brown pigments, 10–150 µm in diameter; parenchymatous cells of cortex, subsquare or polygonal, containing reddish-brown pigments; stone cells, subrounded, subtriangular, subrectangular or irregular shape, some elongated, mostly 14–70 µm in diameter, up to 270 µm in length; fibers mostly in bundles, long fusiform in shape, ends oblique-sharp or blunt-round, with oblique or criss-cross striations, 10–60 µm in diameter; and reticulate and pitted vessels, up to 120 µm in diameter.

- **B. Thin-Layer Chromatography**

  **Standard solution A:** 1.0 mg/mL each of USP Tashinone II₅ RS [1] and USP Salvianolic Acid B RS [2] in methanol

  **Standard solution B:** 50 mg/mL of USP Powdered Chinese Salvia Extract RS [3] in methanol. Sonicate for 15 min, centrifuge, and use the supernatant.

  **Sample solution:** Sonicate about 1.0 g of *Salvia miltiorrhiza* Root and Rhizome Powder in 5.0 mL of methanol for 15 min, centrifuge, and use the supernatant.

  **Chromatographic system**

(See *Chromatography* [621] [4], *Thin-Layer Chromatography.* )
**Adsorbent:** Chromatographic silica gel mixture with an average particle size of 5 µm (HPTLC plates, Si 60 F₂₅₄)

**Application volume:** 5 µL, 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:** Toluene, dichloromethane, ethyl acetate, methanol, and formic acid (4:6:8:1:4)

**Developing distance:** 6 cm

**Derivatization reagent:** 20 mL sulfuric acid in 180 mL methanol

**Analysis**

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

Apply the Samples as bands to a suitable HPTLC plate. Use saturated chambers. Develop the chromatograms in Developing solvent system, remove the plate and dry. Treat with Derivatization reagent, heat for 5 min at 100°, and examine under visible light.

**System suitability:** Under visible light, the chromatogram of Standard solution B exhibits two dark brown and two pink bands in lower half of the chromatogram, with the intense pink band similar in Rₚ and color to the salvianolic acid B band in the chromatogram of Standard solution A. About seven bands in upper half of the chromatogram with increasing Rₚ: a pink, a light purple, a light brown, a purple, a yellow-brown, and an intense purple band similar in Rₚ and color to the tanshinone IIA band in the chromatogram of Standard solution A.

**Acceptance criteria:** Under visible light, the lower half of the chromatogram of the Sample solution exhibits an intense pink band corresponding in color and Rₚ to the band due to salvianolic acid B in the chromatogram of Standard solution A. The upper half of the chromatogram of the Sample solution exhibits an intense purple band close to solvent front corresponding in color and Rₚ to the band due to tanshinone IIA in the chromatogram of Standard solution A. Nine additional bands corresponding to similar bands in the chromatogram of Standard solution B: two dark brown bands and a pale pink band in lower half and a pink, a light purple, a light brown, a purple, and a yellow-brown band in the upper half of the chromatogram of Sample solution.

**C. HPLC**

**Analysis:** Proceed as directed in the Assay for Content of Tanshinones.

**Acceptance criteria:** The chromatogram of the Sample solution exhibits the most intense peak at a retention time corresponding to that of tanshinone IIA in the chromatogram of Standard solution A. The Sample solution chromatogram exhibits two additional peaks corresponding to tanshinone I and cryptotanshinone, of lesser intensity and accounting for about half of the total tanshinones content.

**D. HPLC**

**Analysis:** Proceed as directed in the Assay for Content of Salvianolic Acid B.

**Acceptance criteria:** The chromatogram of the Sample solution exhibits the most intense peak at a retention time corresponding to that of salvianolic acid B in the chromatogram of Standard solution A.

**ASSAY**

**Content of Tanshinones**

**Solution A:** 0.02% Phosphoric acid in water

**Solution B:** Acetonitrile

**Mobile phase:** See Table 1.

---

**Table 1**

<table>
<thead>
<tr>
<th>Mobile phase</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solution A</td>
<td>0.02% Phosphoric acid in water</td>
</tr>
<tr>
<td>Solution B</td>
<td>Acetonitrile</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>See Table 1.</td>
</tr>
<tr>
<td>Time (min)</td>
<td>Solution A (%)</td>
</tr>
<tr>
<td>-----------</td>
<td>----------------</td>
</tr>
<tr>
<td>0</td>
<td>39</td>
</tr>
<tr>
<td>6</td>
<td>39</td>
</tr>
<tr>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>20.5</td>
<td>39</td>
</tr>
<tr>
<td>25</td>
<td>39</td>
</tr>
</tbody>
</table>

[Note—Proceed under subdued light or use low-actinic glassware. The Standard solutions and Sample solution are stable for 24 h at room temperature.]

**Standard solution A:** 0.02 mg/mL of USP Tanshinone IIₐ RS [1] in methanol

**Standard solution B:** 2.0 mg/mL of USP Powdered Chinese Salvia Extract RS [3] in methanol. Sonicate for 15 min, and pass through a membrane filter of 0.45-µm or finer pore size. Discard the first few mL of the filtrate.

**Sample solution:** About 0.3 g of *Salvia miltiorrhiza* Root and Rhizome Powder, accurately weighed, in 40 mL of methanol. Sonicate for 30 min, filter into a 50-mL volumetric flask, and wash the residue and the filter paper with a few mL of methanol. Adjust with methanol to volume, mix, and pass through a membrane filter of 0.45-µm or finer pore size. Discard the first few mL of the filtrate.

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

**Mode:** LC
**Detector:** UV 270 nm
**Column:** 4.6-mm × 25-cm; 5-µm packing L1 (similar to Zorbax Extend C18)
**Column temperature:** 25±1°C
**Flow rate:** 1.0 mL/min
**Injection volume:** 10 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 Powdered Chinese Salvia Extract RS [3] being used.

**Resolution:** NLT 1.5 between the cryptotanshinone and tanshinone I peaks, Standard solution B

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

**Relative standard deviation:** NMT 2.0% determined from the tanshinone IIₐ 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 Powdered Chinese Salvia Extract RS [3] being used, identify the retention times of the peaks corresponding to different tanshinones in the Sample solution chromatogram. The approximate relative retention times of the different peaks for cryptotanshinone, tanshinone I, and tanshinone IIₐ are 0.75, 0.79, and 1.00, respectively.

Separately calculate the percentages of cryptotanshinone, tanshinone I, and tanshinone IIₐ in the
portion of *Salvia miltiorrhiza* Root and Rhizome Powder taken:

\[
\text{Result} = \left( \frac{r_u}{r_s} \right) \times C_s \times \left( \frac{V}{W} \right) \times F \times 100
\]

- \( r_u \) = peak area of the relevant analyte from the *Sample solution*
- \( r_s \) = peak area of tanshinone II from *Standard solution A*
- \( C_s \) = concentration of tanshinone II in *Standard solution A* (mg/mL)
- \( V \) = volume of the *Sample solution* (mL)
- \( W \) = weight of *Salvia miltiorrhiza* Root and Rhizome Powder taken to prepare the *Sample solution* (mg)
- \( F \) = conversion factor for the analytes; 1.18 for crytotanshinone, 1.31 for tanshinone I, and 1.00 for tanshinone II

Calculate the content of tanshinones as the sum of the percentages of cryptotanshinone, tanshinone I, and tanshinone II.

**Acceptance criteria**
- **Tanshinone II**: NLT 0.1% on the dried basis
- **Total tanshinones**: NLT 0.2% on the dried basis

**• CONTENT OF SALVIANOLIC ACID B**

**Solution A:** 0.1% Phosphoric acid in water

**Mobile phase:** *Solution A* and acetonitrile (78:22)

[Note—The *Standard solution* and *Sample solution* are stable for 12 h at room temperature.]

**Solvent:** Methanol and water (8:2)

**Standard solution:** 0.1 mg/mL of USP Salvianolic Acid B RS [2] in Solvent

**Sample stock solution:** About 150 mg of *Salvia miltiorrhiza* Root and Rhizome Powder, accurately weighed, in 40 mL of *Solvent*. Sonicate for 30 min, filter into a 50-mL volumetric flask, and wash the residue and the filter paper with a few mL of *Solvent*. Adjust with *Solvent* to volume, mix, and centrifuge a portion.

**Sample solution:** Dilute a portion of the supernatant from the *Sample stock solution* with *Solvent* (1:2), mix, and pass through a membrane filter of 0.45-µm or finer pore size. Discard the first few mL of the filtrate.

**Chromatographic system**

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

- **Mode:** LC
- **Detector:** UV 286 nm
- **Column:** 4.6-mm × 25-cm; 5-µm packing L1 (similar to Zorbax SB C18)
- **Column temperature:** 25±1°
- **Flow rate:** 1.2 mL/min
- **Injection volume:** 10 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**
- **Tailing factor:** NMT 2.0 for the salvianolic acid B peak
- **Relative standard deviation:** NMT 2.0% determined from the salvianolic acid B peak in repeated injections

**Analysis**
Samples: Standard solution and Sample solution

Using the chromatogram of the Standard solution, identify the retention time of the peak corresponding to salvianolic acid B in the Sample solution chromatogram.

Calculate the percentage of salvianolic acid B in the portion of Salvia miltiorrhiza Root and Rhizome Powder taken:

\[
\text{Result} = \left( \frac{r_u}{r_s} \right) \times C_s \times \left( \frac{V}{W} \right) \times D \times 100
\]

- \( r_u \) = peak area of salvianolic acid B from the Sample solution
- \( r_s \) = peak area of salvianolic acid B from the Standard solution
- \( C_s \) = concentration of salvianolic acid B in the Standard solution (mg/mL)
- \( V \) = volume of the Sample stock solution (mL)
- \( W \) = weight of Salvia miltiorrhiza Root and Rhizome Powder taken to prepare the Sample solution (mg)
- \( D \) = dilution factor to prepare the Sample solution from the Sample stock solution, 2

Acceptance criteria: NLT 3.0% on the dried basis

CONTAMINANTS

- **Elemental Impurities—Procedures <233>** [5]
  - **Acceptance criteria**
    - Arsenic: NMT 2.0 µg/g
    - Cadmium: NMT 0.3 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 <561>: Meets the requirements

- **Microbial Enumeration Tests** [7]<[7.61]> [7]: 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 does not exceed \(10^3\) cfu/g.

- **Tests for Specified Microorganisms** [8]<[8.62] [8]: Meets the requirements of the tests for the absence of Salmonella species and Escherichia coli

SPECIFIC TESTS

- **Articles of Botanical Origin**, [6] Alcohol-Soluble Extractives, Method 1 <561>: NLT 15.0%

- **Articles of Botanical Origin** [6], Water-Soluble Extractives, Method 2 <561>: NLT 35.0%

- **Loss on Drying <731>** [9]
  - **Sample**: 1 g of Salvia miltiorrhiza Root and Rhizome Powder
  - **Analysis**: Dry at 105° for 2 h.
  - **Acceptance criteria**: NMT 13.0%

  - **Sample**: 4 g of Salvia miltiorrhiza Root and Rhizome Powder
  - **Acceptance criteria**: NMT 4.0%

- **Articles of Botanical Origin** [6], Acid-Insoluble Ash <561>
Sample: 4 g of *Salvia miltiorrhiza* Root and Rhizome Powder

Acceptance criteria: NMT 3.0%

**ADDITIONAL REQUIREMENTS**

- **Packaging and Storage:** Preserve in well-closed containers, protected from light and moisture. Store at room temperature.

- **Labeling:** The label states the Latin binomial and the part(s) of the plant from which the article was obtained.

- **USP Reference Standards <11>**
  - USP Powdered Chinese Salvia Extract RS [2]
  - USP Salvianolic Acid B RS [2]
  - USP Tanshinone IIₐ RS [1]

---