Salvia miltiorrhiza Root and Rhizome

Final Authorized Version 1.0

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

SYNONYMS
None Known

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

SELECTED COMMON NAMES
Chinese: 丹参
English: Chinese salvia, Chinese red sage, redroot sage, red-root sage, red sage
Korean: 단삼 (dansam)
Pinyin: Danshen, dan shen, tan shen
Spanish: Racine de Salvia
Swedish: Rödrotssalvia

CONSTITUENTS OF INTEREST
Diterpenoid quinones: Tanshinone I, tashinone IIₐ, and cryptotanshinone
Phenolic acids: Salvianolic acid, lithospermic acid, and rosmarinic acid

IDENTIFICATION
• A. BOTANIC CHARACTERISTICS
  • Macroscopic: Rhizomes are short and thick, sometimes with remains of stems at the apex. Roots, long, cylindrical, slightly curved, some branched, with rootlets, 10-20 cm long, 0.3-1.5 cm in diameter. Externally brownish-red or dark brownish-red, rough, longitudinally wrinkled. The bark of old roots is loose, mostly purplish-brown, usually scaling off; the bark of young roots is closely adhering to wood and
uneasy to be scaled off. Texture hard and fragile, fracture loose, with brownish-red bark and grayish-yellow or purplish-brown wood, showing bundles of vessels, yellowish-white, arranged radially.

**Microscopic**

**Transverse section:** Cork, 4–8 rows of cells with brown contents; rhytidome tissues may be present; cortex broad, parenchyma cells showing reddish-brown granules; phloem narrow, crescent shape; cambium in a ring; xylem vessels, lignified, mainly scalariform and reticulate, numerous near the cambium ring and fewer near the pith; xylem fibers in bundle, scattered radially; pith in the center.

**B. Thin-Layer Chromatography**

**Standard solution A:** 1.0 mg/mL each of USP Tashinone II₄ 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, finely powdered, in 10 mL of methanol for 10 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 distance:** 6 cm

**Developing solvent system:** Toluene, dichloromethane, ethyl acetate, methanol, and formic acid (4:6:8:1:4)

**Derivatization reagent:** Sulfuric acid 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 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 II₄ 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 II₄ in the chromatogram of Standard solution A. Nine additional bands corresponding to similar bands in the chromatogram of Standard solution B: two dark brown 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 the 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>
<thead>
<tr>
<th>Time (min)</th>
<th>Solution A (%)</th>
<th>Solution B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>39</td>
<td>61</td>
</tr>
<tr>
<td>6</td>
<td>39</td>
<td>61</td>
</tr>
<tr>
<td>20</td>
<td>10</td>
<td>90</td>
</tr>
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<td>20.5</td>
<td>39</td>
<td>61</td>
</tr>
<tr>
<td>25</td>
<td>39</td>
<td>61</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, finely powdered and 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 °  
**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 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 IIₐ from Standard solution A
- \( C_S \) = concentration of tanshinone IIₐ in Standard solution A (mg/mL)
- \( V \) = volume of the Sample solution (mL)
- \( W \) = weight of Salvia miltiorrhiza Root and Rhizome taken to prepare the Sample solution (mg)
- \( F \) = conversion factor for analytes; 1.18 for cryptotanshinone, 1.31 for tanshinone I, and 1.00 for tanshinone IIₐ

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

Acceptance criteria

- Tanshinone IIₐ: 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, finely powdered and 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>, *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 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 Standard solution (mg/mL)
- \( V \) = volume of the Sample stock solution (mL)
- \( W \) = weight of *Salvia miltiorrhiza* Root and Rhizome 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>**

  **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** <61>, *General Method for Pesticide Residues Analysis* <561>: Meets the requirements

- **Articles of Botanical Origin** <61>, *Test for Aflatoxins* <561>: Meets the requirements

- **Microbial Enumeration Tests** <71>, *General Methods for Antibiotic Assay* <561>: The total aerobic bacterial count does not exceed \( 10^5 \) cfu/g, the total combined molds and yeasts count does not exceed \( 10^5 \) cfu/g, and the bile-tolerant Gram-
negative bacteria does not exceed $10^3$ cfu/g.

• **Tests for Specified Microorganisms <[b]62> [a]**: Meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli*.

**Specific Tests**

- **Articles of Botanical Origin [6]**, *Foreign Organic Matter* <561>: NMT 2.0%
- **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, finely powdered
  - **Analysis**: Dry at 105° for 2 h.
  - **Acceptance criteria**: NMT 13.0%
- **Articles of Botanical Origin [6]**, *Total Ash* <561>
  - **Sample**: 4 g of *Salvia miltiorrhiza* Root and Rhizome, finely powdered
  - **Acceptance criteria**: NMT 4.0%
- **Articles of Botanical Origin [6]**, *Acid-Insoluble Ash* <561>
  - **Sample**: 4 g of *Salvia miltiorrhiza* Root and Rhizome, finely powdered
  - **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 contained in the article.
- **USP Reference Standards <11> [10]**
  - USP Powdered Chinese Salvia Extract RS [3]
  - USP Salvianolic Acid B RS [12]
  - USP Tanshinone IIa RS [13]