Salvia miltiorrhiza Root and Rhizome

Final Authorized Version 1.0

Salvia miltiorrhiza Root and Rhizome

**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 II<sub>A</sub>; NLT 0.2% of total tanshinones calculated as the sum of cryptotanshinone, tanshinone I, and tanshinone II<sub>A</sub>; 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 II<sub>A</sub>, 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 IIₐ 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 Rf 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 Rf: a pink, a light purple, a light brown, a purple, a yellow-brown, and an intense purple band similar in Rf 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 Rf 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 Rf 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 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 II\textsubscript{A} 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>
<tr>
<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 II\textsubscript{A}, RS\textsuperscript{[1]} in methanol

**Standard solution B:** 2.0 mg/mL of USP Powdered Chinese Salvia Extract RS\textsuperscript{[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>\textsuperscript{[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 II₂₄ peak, *Standard solution A*

**Relative standard deviation:** NMT 2.0% determined from the tanshinone II₂₄ 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 Sample solution* 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 II₂₄ are 0.75, 0.79, and 1.00, respectively.

Separately calculate the percentages of cryptotanshinone, tanshinone I, and tanshinone II₂₄ 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 II₂₄ from *Standard solution A*
- \( C_S \) = concentration of tanshinone II₂₄ 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 II₂₄

Calculate the content of tanshinones as the sum of the percentages of cryptotanshinone, tanshinone I, and tanshinone II₂₄.

**Acceptance criteria**

- **Tanshinone II₂₄:** 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
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**
  - General Method for Pesticide Residues Analysis <561>: Meets the requirements
  - Test for Aflatoxins <561>: Meets the requirements

- **Microbial Enumeration Tests**
  - 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 <[62]> [8]:** 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]