Salvia miltiorrhiza Root and Rhizome

Proposed For Comment Version 0.2

Salvia miltiorrhiza Root and Rhizome

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

**SYNONYMS**
None Known

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

**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 IIβ, and cryptotanshinone
- **Phenolic acids:** Salvianolic acid, lithospermic acid, and rosmarinic acid

**IDENTIFICATION**
- **A. Botanic Characteristics**
  - **Macroscopic:** Rhizomes 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:** 0.5 mg/mL of USP Tashinone IIₐ RS and 1.5 mg/mL USP Salvianolic Acid B RS in alcohol

**Standard solution B:** 50 mg/mL of USP Powdered Chinese Salvia Extract RS in alcohol. 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 5.0 mL of alcohol for 15 min, centrifuge, and use the supernatant.

**Chromatographic system**

(See Chromatography <621>, Thin-Layer Chromatography.)

- **Adsorbent:** Chromatographic silica gel mixture with an average particle size of 5 µm (HPTLC plates)
- **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 A:** Ethyl acetate, chloroform, toluene, formic acid, and methanol (8:6:4:4:1)
- **Developing solvent system B:** Solvent hexane and ethyl acetate (4:1)

**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 A until the solvent front has moved up about 40% of the plate. Remove the plate and allow to dry. Develop the chromatograms in Developing solvent system B until the solvent front has moved up about three-fourths of the plate. Remove the plate and dry. Examine under visible light, and under UV light at 366 nm and 254 nm.

**System suitability:** Under visible light, the chromatogram of Standard solution B exhibits a pink band in the upper-third section similar in *R*ₚ and color to the tanshinone IIₐ band in the chromatogram of Standard solution A, a yellowish-orange band below the pink band, and an orange band at about the middle of the chromatogram due to tanshinone I and cryptotanshinone, respectively.

Under UV light at 366 nm, the chromatogram of Standard solution B exhibits three blue fluorescent bands: an intense band in the lower-third section of the chromatogram corresponding in *R*ₚ and color to the salvianolic acid B band in the chromatogram of Standard solution A; and two minor bands in the lower-third section of the chromatogram and above the salvianolic acid B band, due to lithospermic acid and rosmarinic acid.

Under UV light at 254 nm, the chromatogram of Standard solution B exhibits intense quenching bands at an *R*ₚ corresponding to those for tanshinone IIₐ and salvianolic acid B in the chromatogram of Standard solution A.

**Acceptance criteria:** Under visible light, the chromatogram of the Sample solution exhibits a pink band corresponding in color and *R*ₚ to the band due to tanshinone IIₐ in the chromatogram of Standard solution A, and the following bands corresponding to similar bands in the chromatogram of Standard solution B: a yellowish-orange band in the upper-third section of the chromatogram, and an orange band at about the middle of the chromatogram.
Under UV light at 366 nm, the chromatogram of the Sample solution exhibits an intense blue fluorescent band corresponding in color and $R_f$ to the band due to salvianolic acid B in the chromatogram of Standard solution A, and the following bands corresponding to similar bands in the chromatogram of Standard solution B: two minor blue fluorescent bands in the lower-third section of the chromatogram and above the salvianolic acid B band.

Under UV light at 254 nm, the chromatogram of the Sample solution exhibits two intense quenching bands corresponding in $R_f$ to the bands due to tanshinone II$_A$ and salvianolic acid B in the chromatogram of Standard solution A.

**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$_A$ 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$_A$ RS in methanol

**Standard solution B:** 2.0 mg/mL of USP Powdered Chinese Salvia Extract RS in methanol. Sonicate for 15 min, and pass through a membrane filter having a 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 having a 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 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 being used.

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

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

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

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

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

**Acceptance criteria**

**Tanshinone IIα:** 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 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 having a 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:  

  \[ \text{Result} = \left( \frac{r_U}{r_S} \right) \times C_S \times \frac{V}{W} \times D \times 100 \]  

  \( r_U \) = 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  

  **Acceptance criteria:** NLT 3.0% on the dried basis
CONTAMINANTS

• **Elemental Impurities—Procedures** &lt;233&gt;

  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** &lt;561&gt;: Meets the requirements

• **Articles of Botanical Origin, Test for Aflatoxins** &lt;561&gt;: Meets the requirements

• **Microbial Enumeration Tests** &lt;61&gt;: 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** &lt;2022&gt;: Meets the requirements of the tests for the absence of Salmonella species and Escherichia coli.

SPECIFIC TESTS

• **Articles of Botanical Origin, Foreign Organic Matter** &lt;561&gt;: NMT 2.0%

• **Articles of Botanical Origin, Alcohol-Soluble Extractives, Method 1** &lt;561&gt;: NLT 15.0%

• **Articles of Botanical Origin, Water-Soluble Extractives, Method 2** &lt;561&gt;: NLT 35.0%

• **Loss on Drying** &lt;731&gt;

  Analysis: Dry 1 g of *Salvia miltiorrhiza* Root and Rhizome, finely powdered, at 105°C for 2 h.
  Acceptance criteria: NMT 13.0%

• **Articles of Botanical Origin, Total Ash** &lt;561&gt;

  Analysis: 4 g of *Salvia miltiorrhiza* Root and Rhizome, finely powdered
  Acceptance criteria: NMT 4.0%

• **Articles of Botanical Origin, Acid-Insoluble Ash** &lt;561&gt;

  Analysis: 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** &lt;11&gt;

  - USP Aflatoxins RS [1]
  - USP Powdered Chinese Salvia Extract RS [2]
  - USP Salvianolic Acid B RS [3]
  - USP Tanshinone IIa RS [4]