Salvia miltiorrhiza Root and Rhizome Powdered Extract

Proposed For Comment Version 0.2

Salvia miltiorrhiza Root and Rhizome Powdered Extract

DEFINITION
The article is prepared from the dried roots and rhizomes of Salvia miltiorrhiza Bunge (Family Lamiaceae), collected in spring or fall, by extraction with hydroalcoholic mixtures. The ratio of starting crude plant material to Powdered Extract is 10:1. It contains NLT 90.0% and NMT 110.0% of the labeled amount of tanshinones, calculated as the sum of cryptotanshinone, tanshinone I, and tanshinone IIa; and NLT 90.0% and NMT 110.0% of the labeled amount of salvianolic acid B; all calculated on the dried basis.

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

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

IDENTIFICATION
• A. Thin-Layer Chromatography
  Standard solution A: 0.5 mg/mL of USP Tashinone IIa RS and 1.5 mg/mL of 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: 50 mg/mL of Powdered Extract in alcohol. Sonicate 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 in the upper-third section a pink band similar in Rf and color to the tanshinone IIa band in the chromatogram of Standard solution A, a yellowish-orange band due to tanshinone I below the pink band, and an orange band due to cryptotanshinone at about the middle of the chromatogram.

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 Rf 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 Rf 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 Rf 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 Rf 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 Rf to the bands due to tanshinone IIa and salvianolic acid B in the chromatogram of Standard solution A.

B. 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.

C. 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>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 IIₐ 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:** 2.0 mg/mL of Powdered Extract 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.

**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

[Note—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.]

**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 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 being used, identify the retention times of the peaks corresponding to different tanshinones in the *Sample solution* chromatogram. Separately calculate the percentages of cryptotanshinone, tanshinone I, and tanshinone II_A in the portion of Powdered Extract taken:

\[
\text{Result} = \left( \frac{r_u}{r_S} \right) \times \left( \frac{C_S}{C_U} \right) \times F \times 100
\]

- \(r_u\) = peak area of the relevant analyte from the *Sample solution*
- \(r_S\) = peak area of tanshinone II_A from *Standard solution A*
- \(C_S\) = concentration of tanshinone II_A in *Standard solution A* (mg/mL)
- \(C_U\) = concentration of Powdered Extract in the *Sample solution* (mg/mL)
- \(F\) = conversion factor for analytes; 1.18 for cryptotanshinone, 1.31 for tanshinone I, and 1.00 for tanshinone II_A

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

Calculate the percentage of the labeled amount of tanshinones in the Powdered Extract:

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

- \(P\) = content of tanshinones as determined above (%)
- \(L\) = labeled amount of tanshinones (%)

**Acceptance criteria:** 90.0%–110.0% on the dried basis

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**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 solution:** 2 mg/mL of Powdered Extract 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.

**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 Powdered Extract taken:

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

- \( r_u \) = peak area of salvianolic acid B from the Sample solution
- \( r_s \) = peak area of salvianolic acid B from Standard solution A
- \( C_s \) = concentration of salvianolic acid B in Standard solution A (mg/mL)
- \( C_u \) = concentration of Powdered Extract in the Sample solution (mg/mL)

Calculate the percentage of the labeled amount of salvianolic acid B in the Powdered Extract taken:

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

- \( P \) = content of salvianolic acid B as determined above (%)
- \( L \) = labeled amount of salvianolic acid B (%)

Acceptance criteria: 90.0%–110.0% 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

- **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 \( 10^2 \) cfu/g.

- **Tests for Specified Microorganisms <2022>:** Meets the requirements of the tests for the absence of Salmonella species and Escherichia coli

SPECIFIC TESTS

- **Loss on Drying <731>**
  
  Analysis: Dry 1 g of Powdered Extract at 105° for 5 h.
  
  Acceptance criteria: NMT 8.0%

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