Panax ginseng Steamed Root and Rhizome

**Final Authorized Version 1.0**

*Panax ginseng* Steamed Root and Rhizome

**DEFINITION**

The article consists of the dried root and rhizome of cultivated *Panax ginseng* C.A.Mey. (Family Araliaceae) collected in the fall, steamed until the material is externally reddish-brown and translucent. It contains NLT 1.0% of total ginsenosides, calculated as the sum of ginsenoside Rg₁ (C₄₂H₇₂O₁₄), ginsenoside Re (C₄₈H₈₂O₁₈), ginsenoside Rf (C₄₂H₇₂O₁₄), ginsenoside Rb₁ (C₅₄H₹₂O₂₃), ginsenoside Ro (C₅₁H₷₆O₁₉), ginsenoside Rc (C₅₃H₹₀O₂₂), ginsenoside Rb₂ (C₅₃H₹₀O₂₂), and ginsenoside Rd (C₄₈H₸₂O₁₈), on the dried basis.

**SYNONYMS**

*Aralia ginseng* (C.A.Mey.) Baill.
*Aralia quinquefolia* var. *ginseng* (C.A.Mey.) auct.
*Panax chin-seng* Nees
*Panax quinquefolius* var. *ginseng* (C.A.Mey.) Regel & Maack
*Panax schin-seng* var. *coraiensis* T.Nees
*Panax verus* Oken

**POTENTIAL CONFOUNDING MATERIALS**

Panax ginseng root
Panax notoginseng root
Panax quinquefolius root

**SELECTED COMMON NAMES**

Chinese: 红参 (Hong Shen)
Danish: Rød Ginseng
Japanese: 紅参
Korean: 홍삼

**CONSTITUENTS OF INTEREST**

Ginsenosides: Ginsenoside Rg₁, ginsenoside Re, ginsenoside Rf, ginsenoside Rb₁, ginsenoside Ro, ginsenoside Rc, ginsenoside Rb₂, and ginsenoside Rd

**IDENTIFICATION**

• **A. BOTANICAL CHARACTERISTICS**

  **Macroscopic:** The main roots are spindal, cylindrical or flat, squared columnar, 3–10 cm long, and 1–2 cm in diameter. Externally reddish-brown, translucent, occasionally a few dark yellowish-brown patches appear, with longitudinal furrows, wrinkles, and rootlet scars; interrupted indistinct annulations are sometimes observed on the upper part; the lower part bears 2–3 twisted and intersected branches with curved rootlets or rootlet scars. The rhizomes (Lutou) are 1–2 cm long with several depressed-circular stem scars (Luwan), some bear 1–2 whole or broken adventitious roots (Ding). Texture is hard and fragile, fracture is even and horny.

  **Microscopic**

  **Transverse section:** Cork consists of several layers of cells. Phelloderm is narrow. Phloem shows clefts in the outer part; in the inner part, the parenchymatous cells are densely arranged with scattered resin canals containing yellow secretions. Cambium is in a ring. Xylem rays are broad with vessels singly scattered or grouped in interrupted radial arrangement, occasionally accompanied by non-lignified fibers. Parenchymatous cells contain clusters of calcium oxalate.

• **B. HPTLC FOR ARTICLES OF BOTANICAL ORIGIN <203> [1]**

  **Standard solution A:** 0.5 mg/mL of USP Ginsenoside Rg₁ RS [2] in methanol

  **Standard solution B:** 20 mg/mL of USP Powedered Asian Ginseng Extract RS [3] in methanol. Sonicate for 15 min, centrifuge, and use the supernatant.
Sample solution: Sonicate about 1 g of *Panax ginseng* Steamed Root and Rhizome, finely powdered, in 5 mL of alcohol for 15 min, centrifuge, and use the supernatant.

**Chromatographic system**

**Adsorbent:** Chromatographic silica gel mixture with an average particle size of about 5 µm  
**Application volume:** 2 µL, as 8-mm bands  
**Relative humidity:** Condition the plate to a relative humidity of about 33% using a suitable device.  
**Temperature:** About 25°  
**Developing solvent system:** Methylene chloride, anhydrous ethanol, and water (60: 45: 6.5)  
**Developing distance:** 6 cm  
**Derivatization reagent:** 10% sulfuric acid in alcohol. [Note—Slowly add sulfuric acid to ice-cold ethanol.]

### Analysis

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

Apply the Samples as bands and dry in air. Develop in a saturated chamber, remove the plate from the chamber, and dry in air. Treat the plate with Derivatization reagent, heat at 105° for 5 min, and examine under visible light and UV light at 366 nm.

**System suitability:** Under visible light, Standard solution B exhibits: in the lower-third section, five reddish-violet bands, the above three due to ginsenosides Rb₁, and Rg₁ (co-elute), ginsenoside Rb₂, and ginsenoside Rc in order of increasing Rf; in the middle-third section, three reddish-violet bands due to ginsenoside Rd, ginsenoside Re, and ginsenoside Rf in order of increasing Rf; and in the upper-third section, a most intense reddish-violet band corresponding in Rf and color to the band of ginsenoside Rg₁ in Standard solution A.

Under UV light at 366 nm, Standard solution B exhibits: in the lower-third section, five blue fluorescent bands, the above three due to ginsenosides Rb₁, and Rg₁ (co-elute), ginsenoside Rb₂, and ginsenoside Rc in order of increasing Rf; in the middle-third section, three bands due to ginsenoside Rd (blue fluorescent), ginsenoside Re (pinkish-violet), and ginsenoside Rf (pinkish-violet) in order of increasing Rf; and in the upper-third section, a most intense pinkish-violet band corresponding in Rf and color to the band of ginsenoside Rg₁ in Standard solution A.

**Acceptance criteria:** Under visible light, the Sample solution exhibits: in the lower-third section, three reddish-violet bands corresponding in Rf and color to the bands due to ginsenosides Rb₁, and Rg₁ (co-elute), ginsenoside Rb₂, and ginsenoside Rc (distinction from *Panax notoginseng* Root and Rhizome) in Standard solution B; in the middle-third section, three reddish-violet bands corresponding in Rf and color to the bands due to ginsenoside Rd, ginsenoside Re, and ginsenoside Rf (distinction from *Panax quinquefolius* Root and Rhizome) in Standard solution B; and in the upper-third section, a most intense reddish-violet band corresponding in Rf and color to the band of ginsenoside Rg₁ in Standard solution A.

Under UV light at 366 nm, the Sample solution exhibits: in the lower-third section, three blue fluorescent bands corresponding in Rf and color to the bands due to ginsenosides Rb₁, and Rg₁ (co-elute), ginsenoside Rb₂, and ginsenoside Rc (distinction from *Panax notoginseng* Root and Rhizome) in Standard solution B; in the middle-third section, three bands corresponding in Rf and color to the bands due to ginsenoside Rd, ginsenoside Re, and ginsenoside Rf (distinction from *Panax quinquefolius* Root and Rhizome) in Standard solution B; and in the upper-third section, a most intense pinkish-violet band corresponding in Rf and color to the band of ginsenoside Rg₁ in Standard solution A and Standard solution B.

### HPLC

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

**Acceptance criteria:** The chromatogram of the Sample solution exhibits a peak with a retention time corresponding to ginsenoside Rg₁ in Standard solution A and peaks for ginsenoside Re, ginsenoside Rf (distinction from *Panax quinquefolius* Root and Rhizome and *Panax notoginseng* Root and Rhizome; they do not contain ginsenoside Rf), ginsenoside Rb₁, ginsenoside Ro, ginsenoside Rc (distinction from *Panax notoginseng* Root and Rhizome, which does not contain ginsenosides Ro, Rc, and Rb₁), and ginsenoside Rd corresponding to the retention times for the same ginsenosides in Standard solution B. The peak area ratio for ginsenoside Rb₁ to ginsenoside Rb₂ is NMT 1.0. The intensity of the peak between ginsenoside Ro and ginsenoside Rc is much smaller than that for the ginsenoside Rc peak; the intensity for the peaks of ginsenoside Rg₁ and ginsenoside Rb₂ is similar and most intense in the HPLC chromatogram; the peak intensity of ginsenoside Ro is similar or smaller than ginsenoside Rb₂ (distinction from Standard solution B which represents *Panax ginseng* Root and Rhizome, in which the peak between ginsenoside Ro and ginsenoside Rc is clearly observed; and the peak intensity of ginsenoside Rb₂ is smaller than ginsenoside Ro).

### ASSAY

**CONTENT OF GINSESONIDES**

- **Solution A:** 0.01% 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>82</td>
<td>18</td>
</tr>
<tr>
<td>25</td>
<td>79</td>
<td>21</td>
</tr>
<tr>
<td>35</td>
<td>72</td>
<td>28</td>
</tr>
<tr>
<td>75</td>
<td>68</td>
<td>32</td>
</tr>
<tr>
<td>75.5</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>80.5</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>81</td>
<td>82</td>
<td>18</td>
</tr>
<tr>
<td>88</td>
<td>82</td>
<td>18</td>
</tr>
</tbody>
</table>

Solvent: Methanol and water (7:3)

**Standard solution A**: 0.15 mg/mL of USP Ginsenoside Rg₁, RS [2] in Solvent

**Standard solution B**: 10 mg/mL of USP Powdered Asian Ginseng Extract RS [3] in Solvent. Sonicate and pass through a nylon filter of 0.22-μm pore size before injection.

**Sample solution**: Accurately transfer about 500 mg of Panax ginseng Steamed Root and Rhizome, finely powdered, into a 100-mL glass-stoppered conical flask. Add 25 mL of Solvent, sonicate for 30 min (565 W, 37 KHz), and filter. Repeat the extraction one more time. Rinse the flask and residue left in the flask with 15 mL of Solvent and filter. Combine the extracts and the rinsing. Evaporate under reduced pressure to dryness. Dissolve the residue with Solvent and quantitatively transfer the solution into a 10-mL volumetric flask. Dilute with Solvent to volume and mix. Before injection, pass through a nylon filter of 0.22-μm pore size. Discard the first portion of the filtrate.

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

- **Mode**: LC
- **Detector**: UV 203 nm
- **Column**: 4.6-mm × 15-cm; 2.7-μm packing L1 (similar to Agilent™ C18, 120 Å)
- **Column temperature**: 40°
- **Flow rate**: 1.2 mL/min
- **Injection volume**: 20 µL

**System suitability**

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

**Suitability requirements**

- **Resolution**: NLT 1.5 between ginsenoside Rg₁ and ginsenoside Re peaks, Standard solution B
- **Tailing factor**: NMT 2.0 for the ginsenoside Rg₁ peak, Standard solution A
- **Relative standard deviation**: NMT 2.0% for the ginsenoside Rg₁ peak, Standard solution A
- **Chromatogram similarity**: The chromatogram of Standard solution B is similar to the reference chromatogram provided with the lot of USP Powdered Asian Ginseng Extract RS [3] being used.

**Analysis**

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

[Note—Protect from light. The Standard solutions and Sample solution are stable for 24 h at room temperature.]

Using the chromatograms of Standard solution A, Standard solution B, and the reference chromatogram provided with the lot of USP Powdered Asian Ginseng Extract RS [3] being used, identify the retention times of the peaks corresponding to ginsenoside Rg₁, ginsenoside Re, ginsenoside Rf, ginsenoside Rb₁, ginsenoside Ro, ginsenoside Rc, ginsenoside Rb₂, and ginsenoside Rd in the Sample solution. [Note—See Table 2 for the approximate relative retention times.]

**Table 2**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Approximate Relative Retention Time</th>
<th>Conversion Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ginsenoside Rg₁</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Ginsenoside Re</td>
<td>1.04</td>
<td>1.00</td>
</tr>
</tbody>
</table>
Separately calculate the percentages of ginsenoside Rg\textsubscript{1}, ginsenoside Re, ginsenoside Rf, ginsenoside Rb\textsubscript{1}, ginsenoside Ro, ginsenoside Rc, ginsenoside Rb\textsubscript{2}, and ginsenoside Rd in the portion of Panax ginseng Steamed 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 ginsenoside Rg\textsubscript{1} from Standard solution A
- \( C_S \) = concentration of USP Ginsenoside Rg\textsubscript{1} RS \([2]\) in Standard solution A (mg/mL)
- \( V \) = volume of the Sample solution (mL)
- \( W \) = weight of Panax ginseng Steamed Root and Rhizome taken to prepare the Sample solution (mg)
- \( F \) = conversion factor for the analyte (see Table 2)

Calculate the content of total ginsenosides as the sum of ginsenoside Rg\textsubscript{1}, ginsenoside Re, ginsenoside Rf, ginsenoside Rb\textsubscript{1}, ginsenoside Ro, ginsenoside Rc, ginsenoside Rb\textsubscript{2}, and ginsenoside Rd.

Acceptance criteria: NLT 1.0% on the dried basis

CONTAMINANTS
- \textbf{Articles of Botanical Origin} \(<561>\), \([5]\): Limits of Elemental Impurities \([5]\): Meets the requirements
- \textbf{Articles of Botanical Origin} \(<561>\), \([6]\): Pesticide Residue Analysis \([6]\): Meets the requirements
- \textbf{Microbial Enumeration Tests} \(<61>\) \([7]\): The total aerobic bacterial count does not exceed \(10^5\) cfu/g, the total combined molds and yeasts count does not exceed \(10^4\) cfu/g, and the bile-tolerant Gram-negative bacteria count does not exceed \(10^3\) cfu/g.
- \textbf{Tests for Specified Microorganisms} \(<62>\) \([8]\): Meets the requirements of the tests for the absence of \textit{Salmonella} species and \textit{Escherichia coli}

SPECIFIC TESTS
- \textbf{Articles of Botanical Origin} \(<561>\), \([5]\): Methods of Analysis, Foreign Organic Matter \([6]\): NMT 1.0%
- \textbf{Articles of Botanical Origin} \(<561>\), \([6]\): Methods of Analysis, Alcohol-Soluble Extractives, Method 2 \([6]\): NLT 3.0%
- \textbf{Loss on Drying} \(<731>\) \([9]\): Sample: 1.0 g of Panax ginseng Steamed Root and Rhizome, finely powdered
  - Analysis: Dry the Sample at 105°C for 5 h.
  - Acceptance criteria: NMT 12.0%
- \textbf{Articles of Botanical Origin} \(<561>\), \([6]\): Methods of Analysis, Total Ash \([6]\): NMT 5.0%
- \textbf{Articles of Botanical Origin} \(<561>\), \([6]\): Methods of Analysis, Acid-Insoluble Ash \([6]\): NMT 1.0%

ADDITIONAL REQUIREMENTS
- \textbf{Packaging and Storage}: Preserve in well-closed containers, protected from light and moisture, and store at controlled room temperature.
- \textbf{Labeling}: The label states the Latin binomial following the official name of the plant contained in the article.
- \textbf{USP Reference Standards} \(<11>\) \([10]\)
  - USP Ginsenoside Rg, RS \([2]\)
  - USP Powdered Asian Ginseng Extract RS \([3]\)