Panax ginseng Steamed Root and Rhizome Powder

Proposed For Comment Version 0.2

Panax ginseng Steamed Root and Rhizome Powder

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, reduced to a fine or very fine powder. It contains NLT 1.1% of total ginsenosides, calculated as the sum of ginsenoside Rg1 (C42H72O14), ginsenoside Re (C48H82O18), ginsenoside Rf (C42H72O14), ginsenoside Rb1 (C54H92O23), ginsenoside Ro (C48H76O19), ginsenoside Rc (C53H90O22), ginsenoside Rb2 (C53H90O22), and ginsenoside Rd (C48H82O18), on the dried basis.

POTENTIAL CONFOUNDING MATERIALS
Panax ginseng Root
Panax quinquefolius Root
Panax notoginseng Root

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: Yellowish-white
  Microscopic: Resin canal fragments contain yellow masses of secretion. Clusters of calcium oxalate are 20–68 μm in diameter with acute angles. Cork cells are subsquare or polygonal with fine, wavy, sinuous walls in the surface view. Reticulated and scalariform vessels are 10–56 μm in diameter. Abundant in gelatinized starch granules.

• B. HPTLC FOR ARTICLES OF BOTANICAL ORIGIN <203> [1]
  Standard solution A: 0.5 mg/mL of USP Ginsenoside Rg1 RS in methanol
  Standard solution B: Sonicate about 100 mg of USP Powdered Asian Ginseng Extract RS in 10 mL of 20% methanol for 20 min and centrifuge. Transfer 5 mL of the supernatant into a solid phase extraction column containing L1 packing with a sorbent mass-to-column volume ratio of 200 mg/5 mL. The column was previously conditioned as described in the Note. Wash the column with 2 mL of water at a rate of 1 drop/s and discard the water solution. Elute the column with 1.0 mL of methanol, collect the methanol elution into a 1-mL volumetric flask, and mix.
  [Note—Initially condition the solid phase extraction column with 5 mL of methanol and then with 3 mL of water at a rate of 1 drop/s. Do not allow the column to dry.]
Sample solution: Sonicate about 500 mg of Panax ginseng Steamed Root and Rhizome Powder in 10 mL of 20% methanol for 20 min and centrifuge. Transfer 5 mL of the supernatant into a solid phase extraction column containing L1 packing with a sorbent mass-to-column volume ratio of 200 mg/5 mL. The column was previously conditioned as described in the Note under Standard solution B. Wash the column with 2 mL of water at a rate of 1 drop/s and discard the water solution. Elute the column with 1.0 mL of methanol, collect the methanol elution into a 1-mL volumetric flask, and mix.

Chromatographic system

- Adsorbent: Chromatographic silica gel mixture with an average particle size of about 5 µm
- Application volume: 3 µL, as 8-mm bands
- Relative humidity: Condition the plate to a relative humidity of about 33% using a suitable device.
- Temperature: About 25º
- 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 105º 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 Ro (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 Ro (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 Ro (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 and Standard solution B.

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 Ro (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 (blue fluorescent), ginsenoside Re (pinkish-violet), and ginsenoside Rf (pinkish-violet, distinction from Panax quinquefolius Root and Rhizome) in Standard solution B; and in the upper-third section, a most intense
intense pinkish-violet band corresponding in \( R_f \) and color to the band of ginsenoside Rg1 \(^{[2]}\) in Standard solution A and Standard solution B.

**C. 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, ginsenoside Rb2 (distinction from *Panax notoginseng* Root and Rhizome, which does not contain ginsenosides Ro, Rc, and Rb2), and ginsenoside Rd corresponding to the retention times for the same ginsenosides in Standard solution B. 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 Rb1 are similar and most intense in the HPLC chromatogram; the peak intensity of ginsenoside Ro is similar or smaller than ginsenoside Rb1 (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 Rb1 is smaller than ginsenoside Ro).

**ASSAY**

**Content of Ginsenosides**

**Solution A:** 0.01% 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>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 Powder 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, 120Å)
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 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>
<tr>
<td>Ginsenoside Rf</td>
<td>1.75</td>
<td>0.83</td>
</tr>
<tr>
<td>Ginsenoside Rb1</td>
<td>2.37</td>
<td>1.27</td>
</tr>
<tr>
<td>Ginsenoside Ro</td>
<td>2.44</td>
<td>1.03</td>
</tr>
<tr>
<td>Ginsenoside Rc</td>
<td>2.56</td>
<td>1.22</td>
</tr>
<tr>
<td>Ginsenoside Rb2</td>
<td>2.78</td>
<td>1.22</td>
</tr>
<tr>
<td>Ginsenoside Rd</td>
<td>3.23</td>
<td>1.02</td>
</tr>
</tbody>
</table>

Separately calculate the percentages of ginsenoside Rg1, ginsenoside Re, ginsenoside Rf, ginsenoside Rb1, ginsenoside Ro, ginsenoside Rc, ginsenoside Rb2, and ginsenoside Rd in the portion of Panax
ginseng Steamed Root and Rhizome Powder taken:

Result = \( \frac{r_U}{r_S} \times C_s \times \frac{V}{W} \times F \times 100 \)

\( r_U \) = peak area of the relevant analyte from the Sample solution
\( r_S \) = peak area of ginsenoside Rg1 from Standard solution A
\( C_s \) = concentration of USP Ginsenoside Rg1 [2] RS in Standard solution A (mg/mL)
\( V \) = volume of the Sample solution (mL)
\( W \) = weight of Panax ginseng Steamed Root and Rhizome Powder 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 Rg1, ginsenoside Re, ginsenoside Rf, ginsenoside Rb1, ginsenoside Ro, ginsenoside Rc, ginsenoside Rb2, and ginsenoside Rd.

Acceptance criteria: NLT 1.1% on the dried basis

CONTAMINANTS

- **Articles of Botanical Origin <561>** [5], Limits of Elemental Impurities [5]: Meets the requirements
- **Articles of Botanical Origin <561>, Pesticide Residue Analysis [6]**: Meets the requirements
- **Microbial Enumeration Tests <61>**: 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>**: Meets the requirements of the tests for the absence of Salmonella species and Escherichia coli

SPECIFIC TESTS

- **Articles of Botanical Origin <561>, Methods of Analysis, Alcohol-Soluble Extractives, Method 2 [6]**: NLT 3.0%
- **Loss on Drying <731> [9]**
  - Sample: 1.0 g of Panax ginseng Steamed Root and Rhizome Powder
  - Analysis: Dry the Sample at 105° for 5 h.
  - Acceptance criteria: NMT 12.0%
- **Articles of Botanical Origin <561>, Methods of Analysis, Total Ash: [6]** NMT 5.0%
- **Articles of Botanical Origin <561>, Methods of Analysis, Acid-Insoluble Ash [6]**: NMT 1.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage**: Preserve in well-closed containers, protected from light and moisture, and store at controlled room temperature.
- **Labeling**: The label states the Latin binomial following the official name of the plant contained in the article.
- **USP Reference Standards <11> [10]**
  - USP Ginsenoside Rg1 RS [2]
  - USP Powdered Asian Ginseng Extract RS [3]