Panax notoginseng Root and Rhizome Powdered Extract

Proposed For Comment Version 0.2

Panax notoginseng Root and Rhizome Powdered Extract

**DEFINITION**
The article is prepared from the dried roots, rootlets, and rhizomes of *Panax notoginseng* (Burk.) F. H. Chen (Family Araliaceae), collected before flowering in autumn, by extraction with hydroalcoholic mixtures. The ratio of starting crude plant material to Extract is between 10:1 and 5:1. It contains NLT 90.0%–110.0% of the labeled amount of saponins calculated as the sum of notoginsenoside R1, ginsenoside Rg1, ginsenoside Re, ginsenoside Rb1, and ginsenoside Rd, on the dried basis.

**POTENTIAL CONFOUNGING MATERIALS**
*Panax ginseng* roots and rhizomes
*Panax notoginseng* leaves
*Panax notoginseng* flowers
*Panax quinquefolius* roots and rhizomes

**CONSTITUENTS OF INTEREST**
Saponins: Notoginsenoside R1, ginsenoside Rg1, ginsenoside Re, ginsenoside Rb1, and ginsenoside Rd

**IDENTIFICATION**

- **A. Thin-Layer Chromatography**
  
  **Standard solution A:** 0.5 mg/mL of USP Ginsenoside Rg1 RS in methanol
  
  **Standard solution B:** 10 mg/mL of USP *Panax notoginseng* Powdered Extract RS in alcohol. Sonicate for about 10 min, centrifuge, and use the supernatant.
  
  **Sample solution:** 10 mg/mL of *Panax notoginseng* Root and Rhizome Powdered Extract in alcohol. Sonicate for about 10 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:** 2 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:** Chloroform, alcohol, and water (60:45:6.5)
  
  **Developing distance:** 6 cm
  
  **Derivatization reagent:** A solution of 10% sulfuric acid in alcohol. [Note—Prepare fresh. Keep alcohol cold over ice, carefully and gradually add sulfuric acid.]

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

Apply the Samples as bands to a suitable HPTLC plate and dry in air. Develop the chromatograms in a saturated chamber, remove the plate from the chamber, and dry. Treat with Derivatization reagent, heat for 5-10 min at 105°, and examine immediately under visible light and UV light at 366 nm.

System suitability: Under visible light, the chromatogram of Standard solution B exhibits five reddish-violet bands in the following order with increasing $R_f$: a band in the lower-third section of the chromatogram due to ginsenoside Rb1; three less intense bands clearly separated in the middle-third section of the chromatogram due to ginsenoside Rd, ginsenoside Re, and notoginsenoside R1, respectively; and a band at an $R_f$ corresponding to the ginsenoside Rg1 band in the chromatogram of Standard solution A. The two most intense bands in the chromatogram are those of ginsenoside Rb1 and ginsenoside Rg1.

Under UV light at 366 nm, the chromatogram of Standard solution B exhibits bands in the following order with increasing $R_f$: a blue fluorescent band in the lower-third section of the chromatogram due to ginsenoside Rb1; three less intense bands clearly separated in the middle-third section of the chromatogram due to ginsenoside Rd, ginsenoside Re, and notoginsenoside R1, respectively (the band due to ginsenoside Rd is blue fluorescent while the other two bands are pinkish-violet); and a pinkish-violet band at an $R_f$ corresponding to the ginsenoside Rg1 band in the chromatogram of Standard solution A. The two most intense bands in the chromatogram are those of ginsenoside Rb1 and ginsenoside Rg1.

Acceptance criteria: Under visible light, the chromatogram of the Sample solution exhibits five reddish-violet bands corresponding in $R_f$ to similar bands in the chromatogram of Standard solution B. These bands appear in the following order with increasing $R_f$: a band in the lower-third section of the chromatogram due to ginsenoside Rb1; three less intense bands clearly separated in the middle-third section of the chromatogram due to ginsenoside Rd, ginsenoside Re, and notoginsenoside R1, respectively; and a band at an $R_f$ corresponding to the ginsenoside Rg1 band in the chromatogram of Standard solution A. The two most intense bands in the chromatogram are those of ginsenoside Rb1 and ginsenoside Rg1.

Under UV light at 366 nm, the chromatogram of the Sample solution exhibits the following bands, with increasing $R_f$, corresponding to similar bands in the chromatogram of Standard solution B: a blue fluorescent band in the lower-third section of the chromatogram due to ginsenoside Rb1; three less intense bands clearly separated in the middle-third section of the chromatogram due to ginsenoside Rd, ginsenoside Re, and notoginsenoside R1, respectively (the band due to ginsenoside Rd is blue fluorescent while the other two bands are pinkish-violet); a pinkish-violet band at an $R_f$ corresponding to the ginsenoside Rg1 band in the chromatogram of Standard solution A; and two minor bands, due to ginsenoside Rg3 and ginsenoside Rg2, directly below the ginsenoside Rg1 band. The two most intense bands in the chromatogram are those of ginsenoside Rb1 and ginsenoside Rg1.

[Note—Under UV light at 366 nm and in distinction from Panax notoginseng Root and Rhizome, Panax notoginseng leaves and flowers chromatograms exhibit a red band due to chlorophyll at the solvent front and a minor or no bands at the $R_f$ corresponding to ginsenoside Rg1.]

[Note—Under UV light at 366 nm and in distinction from Panax notoginseng Root and Rhizome, the Panax ginseng Root and Rhizome chromatogram lacks the band at the $R_f$ corresponding to notoginsenoside R1 in the chromatogram of Standard solution B, and exhibits a pinkish-violet band at an $R_f$ slightly higher than that of notoginsenoside R1. The band due to ginsenoside Rg1 is not one of the most intense bands in the chromatogram, and the most intense band is detected in the lower-third section of the chromatogram and in the region where the $R_f$ of ginsenoside Rb1 is detected.]

[Note—Under UV light at 366 nm and in distinction from Panax notoginseng Root and Rhizome, the Panax quinquefolius Root and Rhizome chromatogram lacks the band at the $R_f$ corresponding to...
notoginsenoside R1 in the chromatogram of Standard solution B, exhibits a minor band due to ginsenoside Rg1, and the most intense band is detected in the lower-third section of the chromatogram and in the region where the $R_f$ of ginsenoside Rb1 is detected.]

**B. UHPLC**

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

**Acceptance criteria:** The chromatogram of the Sample solution exhibits peaks at the retention times corresponding to the peaks due to notoginsenoside R1, ginsenoside Rg1, ginsenoside Re, ginsenoside Rb1, and ginsenoside Rd in the chromatogram of Standard solution B. The two most abundant peaks in the chromatogram are those of ginsenoside Rg1 and ginsenoside Rb1.

**ASSAY**

**CONTENT OF SAPONINS**

**Solution A:** 0.03% 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>83</td>
<td>17</td>
</tr>
<tr>
<td>2.4</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>3.5</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>4.2</td>
<td>69</td>
<td>31</td>
</tr>
<tr>
<td>5.0</td>
<td>58</td>
<td>42</td>
</tr>
<tr>
<td>5.1</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>6.0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>6.1</td>
<td>83</td>
<td>17</td>
</tr>
<tr>
<td>7.5</td>
<td>83</td>
<td>17</td>
</tr>
</tbody>
</table>

**Solvent:** Methanol and water (7:3)

**Standard solution A:** 0.04 mg/mL of USP Ginsenoside Rg1 RS in methanol

**Standard solution B:** 3.0 mg/mL of USP Panax notoginseng Powdered Extract RS in Solvent. Sonicate for about 10 min, centrifuge, and use the supernatant. Before injection, pass through a PFTE filter having a 0.2-μm pore size, and discard the first portion of the filtrate.

**Sample solution:** 3.0 mg/mL of Panax notoginseng Root and Rhizome Powdered Extract in Solvent. Sonicate for about 10 min, centrifuge, and use the supernatant. Before injection, pass through a PFTE filter having a 0.2-μm pore size, and discard the first portion of the filtrate.

**Chromatographic system**

(See Chromatography <621>, System Suitability.)

**Mode:** UHPLC

**Detector:** UV 203 nm

**Column:** 2.1-mm × 5-cm; 1.7-μm packing L1 (similar to Kinetex™ C18 100 Å)
Column temperature: 30±1°C  
Flow rate: 0.8 mL/min  
Injection volume: 5 µL  

**System suitability**  
**Samples:** Standard solution A and Standard solution B  
**Suitability requirements**  
**Chromatogram similarity:** The chromatogram from Standard solution B is similar to the reference chromatogram provided with the lot of USP Panax notoginseng Powdered Extract RS being used.  
**Resolution:** NLT 1.5 between the 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%, determined from the ginsenoside Rg1 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 Panax notoginseng Powdered Extract RS being used, identify the retention times of the peaks corresponding to different saponins in the Sample solution chromatogram. The approximate relative retention times of the different peaks are provided in Table 2.  
Separately calculate the percentage of each of the saponins in the portion of Panax notoginseng Root and Rhizome Powdered Extract taken.  

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Relative Retention Times</th>
<th>Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Notoginsenoside R1</td>
<td>0.78</td>
<td>1.09</td>
</tr>
<tr>
<td>Ginsenoside Rg1</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Ginsenoside Re</td>
<td>1.06</td>
<td>1.02</td>
</tr>
<tr>
<td>Ginsenoside Rb1</td>
<td>1.78</td>
<td>1.26</td>
</tr>
<tr>
<td>Ginsenoside Rd</td>
<td>2.04</td>
<td>1.03</td>
</tr>
</tbody>
</table>

Result = \( \frac{r_U}{r_S} \times \frac{C_S}{C_U} \times F \times 100 \)  

\( r_U = \) peak response of the analyte from the Sample solution  
\( r_S = \) peak response for ginsenoside Rg1 from Standard solution A  
\( C_S = \) concentration of ginsenoside Rg1 in Standard solution A (mg/mL)  
\( C_U = \) concentration of Panax notoginseng Root and Rhizome Powdered Extract in the Sample solution (mg/mL)  
\( F = \) conversion factors for the analytes as provided in Table 2  

Calculate the content of saponins as the sum of the percentages of notoginsenoside R1, ginsenoside Rg1, ginsenoside Re, ginsenoside Rb1, and ginsenoside Rd.
Calculate the percentage of the labeled amount of saponins in the Extract:

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

$P$ = content of saponins as determined above (%)  
$L$ = labeled amount of saponins (%)  

**Acceptance criteria:** 90.0%–110.0% of the labeled amount of saponins on the dried basis

**CONTAMINANTS**

- **Elemental Impurities—Procedures <233>**
  - **Acceptance criteria**
    - **Arsenic:** NMT 2.0 µg/g
    - **Cadmium:** NMT 1.0 µg/g
    - **Lead:** NMT 5.0 µg/g
    - **Mercury:** NMT 1.0 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 $10^5$ 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 $10^1$ cfu/g.

- **Tests for Specified Microorganisms <62>:** Meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli*.

**SPECIFIC TESTS**

- **Loss on Drying <731>**
  - **Analysis:** Dry 1.0 g of *Panax notoginseng* Root and Rhizome Powdered Extract at 105° for 5 h.
  - **Acceptance criteria:** NMT 5.0%

**ADDITIONAL REQUIREMENTS**

- **Packaging and Storage:** Preserve in well-closed containers, protected from light and moisture, and store at room temperature.

- **Labeling:** The label states the Latin binomial and, following the official name, the part(s) of the plant from which the article was derived. It meets other labeling requirements under *Botanical Extract <565>*.

- **USP Reference Standards <11>**
  - USP Aflatoxins RS [1]
  - USP Ginsenoside Rg1 RS
  - USP *Panax notoginseng* Powdered Extract RS