Panax notoginseng Root and Rhizome

Proposed For Comment Version 0.2

Panax notoginseng Root and Rhizome

DEFINITION
The article consists of the dried roots, rootlets, and rhizomes of Panax notoginseng (Burk.) F. H. Chen (Family Araliaceae) collected before flowering in autumn. It contains NLT 5.0% of saponins calculated as the sum of notoginsenoside R1, ginsenoside Rg1, ginsenoside Re, ginsenoside Rb1, and ginsenoside Rd, on the dried basis.

SYNONYMS
Aralia quinquefolia var. notoginseng Burkill
Panax pseudoginseng var. notoginseng (Burkill) G. Hoo & C. L.Tseng

POTENTIAL CONFOUNDING MATERIALS
Panax ginseng roots and rhizomes
Panax notoginseng leaves
Panax notoginseng flowers
Panax quinquefolius roots and rhizomes

SELECTED COMMON NAMES
Chinese: 三七, 田七
Danish: Sydkinesisk ginseng
English: Notoginseng, tienchi ginseng, san-qi ginseng, sanchi ginseng, south China ginseng
Japanese: サンシチニンジン
Korean: 삼칠삼, 삼칠인삼
Pinyin: San qi, sanqi, tian qi, sanchi

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

IDENTIFICATION
• A. BOTANICAL CHARACTERISTICS
Macroscopic
   Root: Subconical or cylindrical; 1-6 cm long, 1-4 cm in diameter; externally grayish-brown or grayish-yellow; showing interrupted longitudinal wrinkles, scars of rootlets, and scar of the stem at the apex surrounded by irregular-shaped protrusions; texture heavy and compact, fracture grayish-green, yellowish-green or grayish-white.
   Rootlets: Cylindrical or conical, 2-6 cm long, the upper end 0.8 cm in diameter, the lower end 0.3 cm
Rhizomes: Irregular lumps; show several stem scars and annulations; fracture grayish-green or grayish-white in the center and deep green or gray at the margin

Microscopic

Transverse section: Cork consisting of several rows of radially arranged, thin-wall cells; layers of parenchyma cells, cluster crystals of calcium oxalate are rare and mostly distributed in parenchyma close to cork; phloem parenchyma showing resin canals containing yellow masses; cambium in a ring; and xylem broad, vessels 1-2 rows in groups, arranged radially.

B. 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: Sonicate about 1.0 g of Panax notoginseng Root and Rhizome, finely powdered, in 10 mL of alcohol for 15 min, centrifuge, and use the supernatant.

[Note—Sample solution is stable for 6 h at room temperature.]

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 Rf: 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 Rf 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 Rf: 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 Rf 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 Rf to similar bands in the chromatogram of Standard solution B. These
bands appear in the following order of 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 $R_r$ 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 $R_r$, 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 $R_r$ 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 $R_r$ 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 $R_r$ corresponding to notoginsenoside R1 in the chromatogram of Standard solution B and it exhibits a pinkish-violet band at an $R_r$ 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 $R_r$ 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 $R_r$ 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 $R_r$ of ginsenoside Rb1 is detected.]

• C. 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>
<thead>
<tr>
<th>Time (min)</th>
<th>Solution A (%)</th>
<th>Solution B (%)</th>
</tr>
</thead>
</table>

Table 1
<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Solution A (%)</th>
<th>Solution B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
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<td>17</td>
</tr>
<tr>
<td>2.4</td>
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</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 of 0.2-μm pore size, and discard the first portion of the filtrate.

**Sample solution:** Transfer about 0.3 g of *Panax notoginseng* Root and Rhizome (capable of passing through a 250-μm sieve), accurately weighed, to a 50-mL centrifuge tube. Add 10 mL of Solvent and sonicate for 10 min (140W, 42 kHz). Centrifuge and transfer the supernatant to a 25-mL volumetric flask. Repeat the above extraction two more times, each with 5 mL of Solvent. Combine the extracts in the volumetric flask, dilute with Solvent to volume, and mix. Before injection, pass through a PFTE filter of 0.2-μm pore size, and discard the first portion of the filtrate. [Note—Sample solution is stable for 24 h at room temperature.]

**Chromatographic system**

*(See Chromatography <621>, System Suitability.)*

**Mode:** UPHLC

**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°

**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 taken.

### Table 2

<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 = \( \frac{r_u}{r_s} \times C_s \times \frac{V}{W} \times F \times 100 \)

- \( r_u \) = 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)
- \( V \) = volume of the *Sample solution* (mL)
- \( W \) = weight of Panax notoginseng Root and Rhizome taken to prepare the *Sample solution* (mg)
- \( F \) = 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.

**Acceptance criteria:** NLT 5.0% 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 \( 10^3 \) 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, Foreign Organic Matter <561>:** NMT 2.0%

• **Loss on Drying <731>**
  
  **Analysis:** Dry 1.0 g of *Panax notoginseng* Root and Rhizome, finely powdered, at 105° for 5 h.
  
  **Acceptance criteria:** NMT 14.0%

• **Articles of Botanical Origin, Total Ash <561>**
  
  **Analysis:** 4.0 g of *Panax notoginseng* Root and Rhizome, finely powdered
  
  **Acceptance criteria:** NMT 6.0%

• **Articles of Botanical Origin, Acid-Insoluble Ash <561>**
  
  **Analysis:** 4.0 g of *Panax notoginseng* 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, and 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 <11>**
  
  **USP Aflatoxins RS** [1]
  
  **USP Ginsenoside Rg1 RS**
  
  **USP Panax notoginseng Powdered Extract RS**

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