Panax notoginseng Root and Rhizome Dry 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 Dry Extract is between 10:1 and 5:1. It contains NLT 90.0%–110.0% of the labeled amount of total saponins calculated as the sum of notoginsenoside R1, ginsenoside Rg1, ginsenoside Re, ginsenoside Rb1, and ginsenoside Rd, and NLT 10.0% of notoginsenoside R1, on the dried basis.

POTENTIAL CONFOUNDING 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
Amino acid: Dencichine

IDENTIFICATION

• A. THIN-LAYER CHROMATOGRAPHY
  Standard solution A: 0.5 mg/mL of USP Ginsenoside Rg1 RS [1] in methanol
  Standard solution B: 10 mg/mL of USP Panax notoginseng Root and Rhizome Dry Extract RS [2] in alcohol. Sonicate for about 10 min, centrifuge, and use the supernatant.
  Sample solution: 10 mg/mL of Panax notoginseng Root and Rhizome Dry Extract in alcohol. Sonicate for about 10 min, centrifuge, and use the supernatant.

Chromatographic system
(See Chromatography <621> [3], Thin-Layer Chromatography.)
  Adsorbent: Chromatographic silica gel mixture with an average particle size of 5 µm (HPTLC plates, Si 60 F254)
  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: Methylene chloride, dehydrated 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 at 105° for 5 min, 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 due to ginsenoside Rb1; three less intense bands clearly separated in the middle-third section 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 most intense band is that of ginsenoside Rg1, followed in intensity by the band corresponding to ginsenoside Rb1.
  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 due to ginsenoside Rb1; three less intense bands clearly separated in the middle-third section 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 \textit{Standard solution A}. The two most intense bands 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 \textit{Standard solution B}. These bands appear in the following order with increasing \( R_f \): a band in the lower-third section due to ginsenoside \( Rb1 \); three less intense bands clearly separated in the middle-third section 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 \textit{Standard solution A}. The most intense band is that of ginsenoside \( Rg1 \), followed in intensity by the band corresponding to ginsenoside \( Rb1 \).

Under UV light at 366 nm, the chromatogram of the Sample solution exhibits the following bands in the following order with increasing \( R_f \), corresponding to similar bands in the chromatogram of \textit{Standard solution B}: a blue fluorescent band in the lower-third section due to ginsenoside \( Rb1 \); three less intense bands clearly separated in the middle-third section 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 \textit{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 are those of ginsenoside \( Rb1 \) and ginsenoside \( Rg1 \).

**Note—**Under UV light at 366 nm and in distinction from \textit{Panax notoginseng} Root and Rhizome, \textit{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 \textit{Panax notoginseng} Root and Rhizome, the \textit{Panax ginseng} Root and Rhizome chromatogram lacks the band at the \( R_f \) corresponding to notoginsenoside \( R1 \) in the chromatogram of \textit{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 \textit{Panax notoginseng} Root and Rhizome, the \textit{Panax quinquefolius} Root and Rhizome chromatogram lacks the band at the \( R_f \) corresponding to notoginsenoside \( R1 \) in the chromatogram of \textit{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:

**Acceptance criteria:** Proceed as directed in the Assay for Content of Saponins, Procedure 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>Time (min)</td>
<td>Solution A (%)</td>
<td>Solution B (%)</td>
</tr>
<tr>
<td>-----------</td>
<td>----------------</td>
<td>----------------</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 (70:30)

Standard solution A: 0.04 mg/mL of USP Ginsenoside Rg1 RS [1] in methanol

Standard solution B: 3.0 mg/mL of USP Panax notoginseng Root and Rhizome Dry Extract RS [2] in Solvent. Sonicate for about 10 min, centrifuge, and use the supernatant. Before injection, pass through a PTFE filter of 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 Dry Extract in Solvent. Sonicate for about 10 min, centrifuge, and use the supernatant. Before injection, pass through a PTFE filter of 0.2-μm pore size, and discard the first portion of the filtrate.

Chromatographic system

(See Chromatography <621> [3], 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°

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 of Standard solution B is similar to the reference chromatogram provided with the lot of USP Panax notoginseng Root and Rhizome Dry Extract RS [2] being used.

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%, 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 Root and Rhizome Dry Extract RS [2] 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 Dry Extract taken.

Table 2

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Approximate 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 = \( (r_U/r_S) \times (C_S/C_U) \times F \times 100 \)

- \( r_U \) = 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 Dry 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 Dry Extract:

Result = \( (P/L) \times 100 \)

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

**Procedure 2**

Solution A: 0.03% Phosphoric acid in water

Solution B: Acetonitrile

Mobile phase: See Table 3.

<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>12</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>18</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>22</td>
<td>69</td>
<td>31</td>
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<tr>
<td>26</td>
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<tr>
<td>32</td>
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<td>100</td>
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<tr>
<td>33</td>
<td>83</td>
<td>17</td>
</tr>
<tr>
<td>40</td>
<td>83</td>
<td>17</td>
</tr>
</tbody>
</table>

**Standard solution A:** Proceed as directed in Procedure 1.

**Standard solution B:** Proceed as directed in Procedure 1.

**Sample solution:** Proceed as directed in Procedure 1.

**Chromatographic system**

(See Chromatography <621> [3], System Suitability.)

Mode: HPLC

Detector: UV 203 nm

Column: 4.6-mm × 10-cm; 2.6-µm packing L1 (similar to Kinetex™ C18 100 Å)

Column temperature: 30 ± 1°

Flow rate: 1.5 mL/min

Injection volume: 10 µL

**System suitability:** Proceed as directed in Procedure 1.

**Analysis:** Proceed as directed in Procedure 1.

**Acceptance criteria**

- Total saponins: NLT 90.0%-110.0% of the labeled amount on the dried basis
- Notoginsenoside R1: NLT 10% on the dried basis
CONTAMINANTS

- **Elemental Impurities**<sup>4</sup>—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**<sup>5</sup>, *General Method for Pesticide Residues Analysis*<sup>561</sup>: Meets the requirements

- **Articles of Botanical Origin**<sup>5</sup>, *Test for Aflatoxins*<sup>561</sup>: Meets the requirements

- **Microbial Enumeration Tests**<sup>61</sup>: 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**<sup>62</sup>: Meets the requirements of the tests for the absence of *Salmonella* species and *Escherichia coli*.

SPECIFIC TESTS

- **Presence of Dencichine**

  **Standard solution**: 1.0 mg/mL of USP Dencichine RS in 70% methanol

  **Sample solution**: Sonicate 5 mg/mL of *Panax notoginseng* Root and Rhizome Dry Extract in water at 90° for 20 min. Evaporate the extract under reduced pressure to dryness. Reconstitute dried residue with 2 mL of 70% methanol, centrifuge, and use the supernatant.

  **Chromatographic system**
  (See *Chromatography*<sup>621</sup>, Thin-Layer Chromatography.)

  - **Adsorbent**: Chromatographic silica gel mixture with an average particle size of 5 μm (HPTLC plates, Si 60 F<sub>254</sub>)
  - **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**: Propanol, glacial acetic acid, and water (4:1:2)
  - **Developing distance**: 6 cm
  - **Derivatization reagent**: A solution of 2% ninhydrin in dehydrated alcohol

  **Analysis**
  **Samples**: Standard solution and Sample solution

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

  **System suitability**: Under visible light, the chromatogram of the Standard solution exhibits, in the lower-third section, a yellow band due to the dencichine.

  **Acceptance criteria**: Under visible light, the chromatogram of the Sample solution exhibits, in the lower-third section, a yellow band due to dencichine at an R<sub>f</sub> corresponding to the band due to dencichine in the chromatogram of the Standard solution.

- **Loss on Drying**<sup>731</sup>

  **Sample**: 1.0 g of *Panax notoginseng* Root and Rhizome Dry Extract

  **Analysis**: Dry the Sample 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*<sup>565</sup>.

- **USP Reference Standards**<sup>11</sup>
  - USP Aflatoxins RS [6]
  - USP Dencichine RS
  - USP Ginsenoside Rg1 RS [1]
  - USP *Panax notoginseng* Root and Rhizome Dry Extract RS [2]