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▲White Peony Root Powder

DEFINITION

White Peony Root Powder consists of the dried, peeled roots of *Paeonia lactiflora* Pall. (Family *Paeoniaceae*). The roots are collected in summer or autumn and boiled in water, either before or after peeling, sun dried, and reduced to a fine or very fine powder. It contains NLT 3.0% of total benzoyl monoterpenoid glycosides calculated as the sum of albiflorin ($C_{23}H_{28}O_{11}$), paeoniflorin ($C_{23}H_{28}O_{11}$), and benzoylpaeoniflorin ($C_{30}H_{32}O_{12}$) on the dried basis.

IDENTIFICATION

• **A. HPTLC for Articles of Botanical Origin ⟨203⟩**

Standard solution A: 0.25 mg/mL of USP Paeoniflorin RS in methanol

Standard solution B: 30 mg/mL of USP *Paeonia lactiflora* Peeled Root Dry Extract RS in methanol. Sonicate for 15 min, centrifuge, and use the supernatant.

Sample solution: 500 mg of White Peony Root Powder in 5 mL of methanol. Sonicate for 15 min, centrifuge, and use the supernatant.

Chromatographic system

Adsorbent: Silica gel with fluorescence indicator F_{254}, precoated plates, for HPTLC

Application volume: 8 µL as 8-mm bands

Relative humidity: Condition the plate to a relative humidity of about 33% using a suitable device.

Developing solvent system: Methylene chloride, ethyl acetate, methanol, and formic acid (35:5:5:3)

Derivatization reagent: 10% sulfuric acid in alcohol. [Note—Prepare on the day of the test; it can be used for up to 1 week if stored in a tightly closed container in a cool place. Keep alcohol cold over ice, and add sulfuric acid slowly.]

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*, and heat at 100° for 5 min. Immediately examine under long wavelength UV light and under white light.

System suitability

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

Suitability requirements

Under long wavelength UV light: *Standard solution A* exhibits a dark brown band due to paeoniflorin in the lower-third of the chromatogram. *Standard solution B* exhibits a band corresponding in R_F and color to the band due to paeoniflorin in *Standard solution A*, a blue band due to albiflorin below the paeoniflorin band, and a dark band due to gallic acid above the paeoniflorin band. In the upper half of the chromatogram, *Standard solution B* exhibits bands in the following order with increasing R_F: a pinkish-violet band, a light dark band, a narrow violet band, a pinkish-violet band, and a broad purple band.

Under white light: *Standard solution A* exhibits a brown band due to paeoniflorin in the lower third of the chromatogram. *Standard solution B* exhibits a band corresponding in R_F and color to the band due to paeoniflorin in *Standard solution A*, a brown band due to albiflorin below the paeoniflorin band, and a brown band due to gallic acid above the paeoniflorin band. In the upper half of the chromatogram, *Standard solution B* exhibits brown bands in the following order with increasing R_F: three well-separated bands, a purple brown band, and a reddish brown band.

Acceptance criteria

Under long wavelength UV light: The *Sample solution* exhibits the following bands corresponding to similar bands in *Standard solution B*. In the lower third of the chromatogram, the *Sample solution* exhibits a dark brown band corresponding in R_F and color to the band due to paeoniflorin in *Standard solution A*; a blue band due to albiflorin below the paeoniflorin band (a distinction from the bark of *Paeonia × suffruticosa*, the unpeeled root of *Paeonia lactiflora*, and the root of *Paeonia anomala* subsp. *veitchii*); and a dark band due to gallic acid

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above the paeoniflorin band. In the upper half of the chromatogram, the *Sample solution* exhibits several bands in the following order with increasing R_F: a pinkish-violet band, a light dark band, a narrow violet band, a pinkish-violet band, and a broad purple band.

Under white light: The *Sample solution* exhibits the following bands corresponding to similar bands in *Standard solution B*. In the lower third of the chromatogram, the *Sample solution* exhibits a brown band corresponding in R_F and color to the band due to paeoniflorin in *Standard solution A*; a brown band due to albiflorin below the paeoniflorin band (a distinction from the bark of *Paeonia × suffruticosa*, the unpeeled root of *Paeonia lactiflora*, and the root of *Paeonia anomala* subsp. *veitchii*); and a brown band due to gallic acid above the paeoniflorin band. In the upper half of the chromatogram, the *Sample solution* exhibits several brown bands in the following order with increasing R_F: three well-separated bands, a purple brown band, and a reddish brown band.

• **B. HPLC**

Analysis: Proceed as directed in the test for *Content of Benzoyl Monoterpenoid Glycosides*.

Acceptance criteria: The *Sample solution* exhibits the most-intense peak at the retention time corresponding to paeoniflorin in *Standard solution A* and *Standard solution B*. The *Sample solution* also exhibits peaks due to albiflorin (a distinction from the bark of *Paeonia × suffruticosa* and the root of *Paeonia anomala* subsp. *veitchii*) and benzoylpaeoniflorin at the retention times corresponding to the same constituents in *Standard solution B*. No significant peak corresponding to paeonol appears after the benzoylpaeoniflorin peak (a distinction from the bark of *Paeonia × suffruticosa* and the unpeeled root of *Paeonia lactiflora*). No peak corresponding to paeoniflorin sulfonate is observed at a relative retention time of about 0.45 with respect to paeoniflorin (a distinction from sulfur-fumigated White Peony Root). The content ratios of albiflorin and benzoylpaeoniflorin relative to paeoniflorin are within the ranges in *Table 2*.

COMPOSITION

• **Content of Benzoyl Monoterpenoid glycosides**

Solution A: 0.10% phosphoric acid in water

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	87	13
4	86	14
7	84	16
15	79	21
25	36	64
25.1	5	95
30	5	95
30.1	87	13
35	87	13

Solvent: Methanol and water (1:1)

Standard solution A: 0.12 mg/mL of USP Paeoniflorin RS in *Solvent*

Standard solution B: 2 mg/mL of USP *Paeonia lactiflora* Peeled Root Dry Extract RS in *Solvent*. Sonicate for 10 min, centrifuge, and pass through a suitable membrane filter of 0.45-µm or finer pore size.

Sample solution: Transfer about 40 mg of White Peony Root Powder, accurately weighed, into a suitable flask. Add 10.0 mL of *Solvent*, and close tightly. Weigh the filled flask accurately, and sonicate for 30 min. Cool to room temperature, and adjust to the initial weight by adding *Solvent*, if needed. Before injection, pass through a suitable membrane filter of 0.45-µm or finer pore size, and discard the first portion of the filtrate.

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

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