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Valerian Tincture

DEFINITION
Valerian Tincture is prepared as follows.

Valerian	200 g
A mixture of Alcohol and Water (6:4) to (8:2), a sufficient quantity to make	1000 mL

Prepare the Tincture as directed for [Botanical Extracts \(565\)](#), [Tinctures](#), [Maceration Process](#). Valerian Tincture contains NLT 0.015% of valerenic acids, calculated as the sum of hydroxyvalerenic acid, acetoxyvalerenic acid, and valerenic acid.

IDENTIFICATION

• **A. THIN-LAYER CHROMATOGRAPHY**

- Standard solution A:** 0.25 mg/mL of [USP Valerenic Acid RS](#) in methanol
- Standard solution B:** 40 mg/mL of [USP Powdered Valerian Extract RS](#) in methanol. Sonicate for 10 min, centrifuge, and use the supernatant.
- Sample solution:** Centrifuge the Tincture, and use the supernatant.
- Chromatographic system**
- Adsorbent:** Chromatographic silica gel mixture with an average particle size of 2–10 µm (HPTLC plates)
- Application volume:** 5 µL of *Standard solution A* and *Standard solution B* and 10 µL of the *Sample solution*, as 8-mm bands
- Developing solvent system:** A mixture of cyclohexane, ethyl acetate, and glacial acetic acid (60:38:2)
- Derivatization reagent A:** A mixture of glacial acetic acid and hydrochloric acid (1:4)
- Derivatization reagent B:** 0.5 mL of *p*-anisaldehyde, 10 mL of glacial acetic acid, and 5 mL of sulfuric acid. Add to 85 mL of ice-cold methanol, and mix.

Analysis

- Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*
- Apply the *Samples* as bands to a suitable high-performance thin-layer chromatographic plate. Use a saturated chamber, and condition the plate to a relative humidity of about 33% using a suitable device. Develop the chromatograms over a distance of 6 cm. Remove the plate from the chamber, dry, derivatize with *Derivatization reagent A*, heat at 120° for 5 min, and examine under white light. Derivatize with *Derivatization reagent B*, heat at 100° for 3 min, and examine under white light.
- Acceptance criteria:** After treatment with *Derivatization reagent A* and heating, the *Sample solution* does not exhibit an intense blue band at about the middle of the chromatogram nor any other significant bands [distinction from Mexican valerian (*Valeriana edulis*)], though minor bands may be observed.
- After treatment with *Derivatization reagent B* and heating, the *Sample solution* exhibits three violet bands in positions and colors similar to the bands of *Standard solution B*. These bands include a minor band in the lower third of the chromatogram due to hydroxyvalerenic acid, a major band at about the middle of the chromatogram due to acetoxyvalerenic acid [distinction from Scouler's valerian (*Valeriana wallichii*)], and a major band at an *R_f* corresponding to the valerenic acid band of *Standard solution A* and *Standard solution B*. Other minor bands may be observed in the *Sample solution* and in *Standard solution B*.

• **B. HPLC**

- Analysis:** Proceed as directed in the test for *Content of Valerenic Acids*.
- Acceptance criteria:** The *Sample solution* exhibits a peak at a retention time corresponding to the valerenic acid peak of *Standard solution A*. The *Sample solution* shows additional peaks corresponding to hydroxyvalerenic acid and acetoxyvalerenic acid.

STRENGTH

• **CONTENT OF VALERENIC ACIDS**

- Solution A:** Mix 6 mL of 85% phosphoric acid with 900 mL of water, dilute with water to 1000 mL, mix, filter, and degas.
- Solution B:** Mix 6 mL of 85% phosphoric acid with 900 mL of methanol, dilute with methanol to 1000 mL, mix, filter, and degas.



Mobile phase: See [Table 1](#).

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	40	60
15	5	95
25	5	95
30	40	60

Solvent: A mixture of methanol and a solution of 0.1% phosphoric acid in water (3:1)
Standard solution A: 0.02 mg/mL of [USP Valerenic Acid RS](#) in methanol. Sonicate if necessary.
Standard solution B: Sonicate a portion of [USP Powdered Valerian Extract RS](#) in *Solvent* to obtain a solution having a concentration of about 20 mg/mL. Before injection, pass through a membrane filter of 0.45-µm or finer pore size, discarding the first few mL of the filtrate.
Sample solution: Use Tincture. Before injection, pass through a membrane filter of 0.45-µm or finer pore size, discarding the first few mL of the filtrate.

Chromatographic system
(See [Chromatography \(621\)](#), *System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 25-cm; end-capped, 5-µm 100 Å packing L1
Column temperature: 40°
Flow rate: 1.0 mL/min
Injection volume: 25 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 Powdered Valerian Extract RS](#) being used.
Tailing factor: NMT 2.0 for the valerenic acid peak, *Standard solution A*
Relative standard deviation: NMT 2.0% for the valerenic acid peak in repeated injections, *Standard solution A*

Analysis
Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*
Identify the valerenic acids in the *Sample solution* chromatogram by comparison with the chromatograms of *Standard solution A*, *Standard solution B*, and the reference chromatogram provided with the lot of [USP Powdered Valerian Extract RS](#) being used.
Calculate the percentage of valerenic acids (sum of hydroxyvalerenic acid, acetoxyvalerenic acid, and valerenic acid) in the portion of Tincture taken:

$$\text{Result} = \{[\sum(r_U \times F)]/r_S\} \times C_S \times 0.1$$

r_U = peak areas of the relevant analytes from the *Sample solution*
 F = conversion factor for each analyte (1.10 for hydroxyvalerenic acid, 1.25 for acetoxyvalerenic acid, and 1.00 for valerenic acid)
 r_S = peak area of valerenic acid from *Standard solution A*
 C_S = concentration of valerenic acid in *Standard solution A* (mg/mL)

Acceptance criteria: NLT 0.015% of valerenic acids, calculated as the sum of hydroxyvalerenic acid, acetoxyvalerenic acid, and valerenic acid

OTHER COMPONENTS
• [ALCOHOL DETERMINATION, Method I \(611\)](#): NLT 90.0% and NMT 110.0% of the labeled amount of alcohol (C₂H₅OH)