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# St. John's Wort Flowering Top Powder

## DEFINITION

St. John's Wort Flowering Top Powder consists of the dried flowering tops of *Hypericum perforatum* L. (Fam. Hypericaceae), gathered shortly before or during flowering, and reduced to a fine or a very fine powder. It contains NLT 0.6% of hyperforin ($C_{35}H_{52}O_4$) and NLT 0.04% of the combined total of hypericin ($C_{30}H_{16}O_8$) and pseudohypericin ($C_{30}H_{16}O_9$), on the dried basis.

## IDENTIFICATION

• **A.** It meets the requirements in *Specific Tests, Botanical Characteristics*.

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

**Standard solution A:** 0.5 mg/mL of USP Rutin RS in methanol

**Standard solution B:** 0.5 mg/mL of USP Hyperoside RS in methanol

**Standard solution C:** 50 mg/mL of USP Powdered St. John's Wort Extract RS in methanol. Sonicate for 20 min, centrifuge, and use the clear supernatant.

**Sample solution:** Sonicate 1 g of St. John's Wort Flowering Top Powder in 10 mL of methanol for about 20 min, centrifuge, and use the clear supernatant.

**Chromatographic system**

**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 33%.

**Temperature:** Ambient, not to exceed 30°

**Developing solvent system:** Ethyl acetate, glacial acetic acid, formic acid, water, and methylene chloride (10:1.0:1.0:1.1:2.5)

**Developing distance:** 6 cm

**Derivatization reagent A:** 5-mg/mL solution of 2-aminoethyl diphenylborinate in ethyl acetate

**Derivatization reagent B:** 10-mg/mL solution of polyethylene glycol 400 in methylene chloride

**Analysis**

**Samples:** *Standard solution A*, *Standard solution B*, *Standard solution C*, 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. Heat the plate at 105° for 3 min, treat while still warm with *Derivatization reagent A*, and dry in air. Then treat with *Derivatization reagent B*, dry in air, and examine under UV light at 365 nm.

**System suitability:** *Standard solution C* exhibits, in the lower third section, two yellowish-orange fluorescent bands corresponding to rutin and hyperoside in *Standard solution A* and *Standard solution B*, respectively; a blue fluorescent band directly below the hyperoside band corresponding to chlorogenic acid; and two red fluorescent bands due to pseudohypericin (lower $R_F$) and hypericin in the upper third section. The bands due to pseudohypericin and hypericin are clearly separated.

**Acceptance criteria:** The *Sample solution* exhibits the following: two yellowish-orange fluorescent bands at $R_F$ corresponding to rutin and hyperoside in *Standard solution A*, *Standard solution B*, and *Standard solution C*; a blue fluorescent band directly below the hyperoside band corresponding to the chlorogenic acid in *Standard solution C*; two red fluorescent bands at $R_F$ corresponding to pseudohypericin and hypericin in *Standard solution C*; and two to three yellowish-orange fluorescent bands in the middle third section corresponding to similar bands in *Standard solution C*.

## COMPOSITION

• **Content of Hypericin and Pseudohypericin**

[Note—Conduct all sample preparations with minimal exposure to subdued light, and use low-actinic glassware.]

**Solvent:** Methanol and acetone (1:1)

**Solution A:** Phosphoric acid and water (3:997)

**Solution B:** Acetonitrile

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**Solution C:** Methanol

**Mobile phase:** See *Table 1*.

**Table 1**

| Time (min) | Solution A (%) | Solution B (%) | Solution C (%) |
|---|---|---|---|
| 0 | 100 | 0 | 0 |
| 10 | 85 | 15 | 0 |
| 30 | 70 | 20 | 10 |
| 40 | 10 | 75 | 15 |
| 55 | 5 | 80 | 15 |
| 56 | 100 | 0 | 0 |
| 66 | 100 | 0 | 0 |

**Standard solution A:** 2.5 µg/mL of USP Oxybenzone RS in *Solvent*

**Standard solution B:** 1 mg/mL of USP Powdered St. John's Wort Extract RS in *Solvent*

**Sample solution:** Accurately weigh about 1 g of St. John's Wort Flowering Top Powder into a round-bottom flask equipped with a condenser and protected from light, add 50 mL of *Solvent* and a magnetic stirring bar, and heat at 60° for 2 h while stirring. Cool to room temperature, and pass through a filter paper into a 50-mL volumetric flask. Wash the flask and the residue on the filter with *Solvent*, and dilute with the washings to volume. Pass the solution through a PTFE membrane filter of 0.45-µm or finer pore size, and use the filtrate.

**Chromatographic system**

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

**Mode:** LC

**Detector:** UV 270 nm and Vis 588 nm

**Columns**

**Guard:** Packing L1

**Analytical:** 4.6-mm × 25-cm; packing L1

**Column temperature:** 30°

**Flow rate:** 1 mL/min

**Injection volume:** 20 µL

**System suitability**

**Samples:** *Standard solution A* (record the peak responses at 270 nm) and *Standard solution B* (record the peak responses at 270 and 588 nm)

**Suitability requirements**

**Chromatogram similarity:** The chromatograms of *Standard solution B* are similar to the respective reference chromatograms provided with the lot of USP Powdered St. John's Wort Extract RS being used.

**Column efficiency:** NLT 100,000 theoretical plates for oxybenzone, *Standard solution A*

**Tailing factor:** NMT 1.5 for oxybenzone, *Standard solution A*

**Relative standard deviation:** NMT 2.0% in replicate injections, *Standard solution A*

**Analysis**

**Samples:** *Standard solution A* and *Sample solution*

Measure the areas of the relevant peaks in the *Sample solution* at 270 nm.

Calculate the combined total of hypericin ($C_{30}H_{16}O_8$) and pseudohypericin ($C_{30}H_{16}O_9$) percentages in the portion of St. John's Wort Flowering Top Powder taken:

$$\text{Result} = (C_S/r_S) \times \Sigma(r_{Ui}/F_i) \times (V/W) \times 100$$

$C_S$ = concentration of USP Oxybenzone RS in *Standard solution A* (mg/mL)

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