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St. John's Wort Flowering Top Dry Extract

DEFINITION

St. John's Wort Flowering Top Dry Extract is prepared from comminuted dried flowering tops of *Hypericum perforatum* L. (Fam. Hypericaceae), gathered shortly before or during flowering, and extracted with 80% methanol or other suitable solvents. It contains NLT 90.0% and NMT 110.0% of the labeled combined total of hypericin ($C_{30}H_{16}O_8$) and pseudohypericin ($C_{30}H_{16}O_9$) and NLT 90.0% and NMT 110.0% of hyperforin ($C_{35}H_{52}O_4$), on the anhydrous basis. It may contain suitable added substances.

IDENTIFICATION

• A. [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: 50 mg/mL of St. John's Wort Flowering Top Dry Extract in [methanol](#), sonicate for 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 of 2-aminoethyl diphenylborinate in [ethyl acetate](#)

Derivatization reagent B: 10 mg/mL 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 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*.

• B. THIN-LAYER CHROMATOGRAPHY

Presence of hyperforin

Standard solution: 50 mg/mL of USP Powdered St. John's Wort Extract RS in [methanol](#). Sonicate for 20 min, and use the clear supernatant.

Sample solution: 50 mg/mL of St. John's Wort Flowering Top Dry Extract in [methanol](#). Sonicate for 20 min, and use the clear supernatant.

Chromatographic system

(See [Chromatography \(621\)](#), [Thin-Layer Chromatography](#).)

Adsorbent: 0.50-mm layer of chromatographic silica gel mixture

Application volume: 10 µL

Developing solvent system: [Solvent hexane](#) and [ethyl acetate](#) (4:1)

Derivatization reagent: Prepare a solution containing 0.38 g of [ceric ammonium sulfate](#) and 3.8 g of [ammonium molybdate](#) in 100 mL of [2 N sulfuric acid](#).

Analysis

Samples: *Standard solution* and *Sample solution*

Develop in a saturated chamber until the solvent front has moved NLT 18 cm, and dry the plate in a current of air. Treat the plate with *Derivatization reagent*, heat at 140° for 15 min, and examine under white light.

Acceptance criteria: The blue zone due to hyperforin at an *R_f* value of about 0.54 in the *Sample solution* corresponds in color and position to that in the *Standard solution*.

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](#)

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: 1 mg/mL of St. John's Wort Flowering Top Dry Extract in a mixture of [methanol](#) and [water](#) (9:1). Sonicate to dissolve, pass through a PTFE 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