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St. John's Wort Flowering Top Dry Extract Capsules

DEFINITION

St. John's Wort Flowering Top Dry Extract Capsules contain St. John's Wort Flowering Top Dry Extract. They contain NLT 90% and NMT 110% of the labeled amount of hypericins, calculated as the sum 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 the labeled amount of hyperforin ($C_{35}H_{52}O_4$).

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: Transfer a portion of the contents of the Capsules, equivalent to 500 mg of St. John's Wort Flowering Top Dry Extract, to a conical flask, add 10 mL of [methanol](#), mix and sonicate for 20 min, centrifuge, and use the 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 366 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. LC

Analysis: Proceed as directed in the *Content of Hypericin, Pseudohypericin, and Hyperforin*.

Acceptance criteria: The chromatogram of the *Sample solution* exhibits peaks at the retention times corresponding to the peaks due to hypericin, pseudohypericin, and hyperforin in the chromatogram of *Standard solution B*.

STRENGTH

• CONTENT OF HYPERICIN, PSEUDOHYPERICIN, AND HYPERFORIN

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



Documents

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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: Determine the total weight of 20 Capsules. Open the Capsules and combine their contents in an appropriate container.

Weigh the empty Capsule shells and calculate the average fill weight per Capsule. Transfer a portion of the Capsule contents, equivalent to 200 mg of St. John's Wort Flowering Top Dry Extract into a 200-mL volumetric flask. Add 150 mL of *Solvent* and sonicate for 30 min with occasional shaking. Cool to room temperature, dilute to volume with *Solvent*, mix well, centrifuge. Before injection, pass through a polytetrafluoroethylene (PTFE) membrane filter of 0.45-µm 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

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*

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.

Analysis

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

Record the peak responses at 270 nm. Using the chromatograms of *Standard solution B*, and the reference chromatogram provided with the lot of USP Powdered St. John's Wort Extract RS being used, identify the peaks corresponding to hypericin, pseudohypericin, and hyperforin in the *Sample solution*. Measure the areas of the relevant peaks in the *Sample solution* chromatogram.