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Powdered Holy Basil Leaf

DEFINITION

Powdered Holy Basil Leaf is the pulverized dried leaf of *Ocimum tenuiflorum* L. (Fam. Lamiaceae). It contains NLT 0.5% of triterpenes, calculated as the sum of oleanolic acid and ursolic acid, on the dried basis.

IDENTIFICATION

• **A.** Powdered Holy Basil Leaf meets the requirements for *Specific Tests, Botanic Characteristics*.

• **B. THIN-LAYER CHROMATOGRAPHY**

Standard solution A: 0.5 mg/mL of [USP Rosmarinic Acid RS](#) in methanol

Standard solution B: 10 mg/mL of [USP Powdered Holy Basil Extract RS](#) in methanol

Sample solution: Sonicate about 1 g of Powdered Holy Basil Leaf in 10 mL of methanol for 10 min, centrifuge, and use the supernatant. [NOTE –Reserve a portion of the supernatant for *Identification* test C.]

Chromatographic system

Adsorbent: Chromatographic silica gel mixture with an average particle size of 5 µm (HPTLC plates)

Application volume: 2 µL of *Standard solution A*, 4 µL of *Standard solution B*, and 8 µL of the *Sample solution* as 8-mm bands

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

Developing solvent system: A mixture of ethyl acetate, formic acid, and water (15:1:1)

Developing distance: 6 cm

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

Derivatization reagent B: 50-mg/mL solution of polyethylene glycol 400 in dichloromethane

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, and dry in air (see [Chromatography \(621\)](#)).

Develop the chromatograms in a saturated chamber, remove the plate from the chamber, dry in air, heat at 100° for 3 min, derivatize the plate while still warm with *Derivatization reagent A*, dry in air, then derivatize with *Derivatization reagent B*, dry in air, and examine under UV light at 366 nm.

System suitability: The chromatogram of *Standard solution B* exhibits, in the upper third section, a blue fluorescent band corresponding to the band due to rosmarinic acid in the chromatogram of *Standard solution A*, and a less intense blue fluorescent band, right above the band due to rosmarinic acid, corresponding to caffeic acid, and a red band above the caffeic band. The bands due to rosmarinic acid and caffeic acid are clearly separated. The chromatogram of *Standard solution B* also shows the most intense band, an orange band, in the lower third section of the chromatogram, and two greenish bands in the middle third section of the chromatogram.

Acceptance criteria: The chromatogram of the *Sample solution* exhibits the following bands corresponding to similar bands in *Standard solution B*: a blue fluorescent band at an R_f corresponding to the rosmarinic acid band; a less intense blue fluorescent band at an R_f above that of the rosmarinic acid band, corresponding to caffeic acid; a red band above the caffeic acid band; and a red band at the solvent front due to chlorophyll; two greenish bands in the middle third section of the chromatogram; and the most intense band, an orange band, with a blue band right above in the lower third section of the chromatogram (distinction from sage leaf, thyme leaf, basil leaf, and oregano leaf, all show two orange bands in the lower third section of the chromatogram; and lemon balm leaf which lacks the band). The *Sample solution* does not exhibit yellowish-orange bands at the middle of the chromatogram (distinction from rosemary leaf and thyme leaf).

• **C. THIN-LAYER CHROMATOGRAPHY**

Standard solution A: 0.2 mg/mL of [USP Ursolic Acid RS](#) in methanol

Standard solution B: 1.0 mg/mL of eugenol in methanol

Standard solution C: 1.0 mg/mL of methyleugenol in methanol

Standard solution D: 10 mg/mL of [USP Powdered Holy Basil Extract RS](#) in methanol

Sample solution: Use the solution prepared in *Identification* test B.

Chromatographic system

Adsorbent: Chromatographic silica gel mixture with an average particle size of 5 µm (HPTLC plates)

Application volume: 2 µL of *Standard solution A*, *Standard solution B*, and *Standard solution C*; 4 µL of *Standard solution D*, and 8 µL of the *Sample solution* as 8-mm bands

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

Developing solvent system: A mixture of toluene and ethyl acetate (85:15)

Developing distance: 6 cm

Derivatization reagent: Place 85 mL of methanol in a 100-mL glass bottle, and cool it down in a water–ice cubes–salt bath or in a freezer. To the ice-cold methanol, add 10 mL of acetic acid and 5 mL of sulfuric acid, slowly and carefully, and mix well. Allow the mixture to cool to room temperature, and then add 0.5 mL of *p*-anisaldehyde.

Analysis

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

Apply the *Samples* as bands to a suitable high performance thin-layer chromatographic plate, and dry in air (see [Chromatography \(621\)](#)).

Develop the chromatograms in a saturated chamber, remove the plate from the chamber, dry in air, derivatize with *Derivatization reagent*, heat at 100° for 3–5 min, set aside to cool, and examine under visible light.

System suitability: *Standard solution A* exhibits a violet band. *Standard solution B* and *Standard solution C* each exhibit a greenish band. *Standard solution D* exhibits a purple band above the green band corresponding to eugenol or methyleugenol in *Standard solution B* or *Standard solution C*.

Acceptance criteria: The chromatogram of the *Sample solution* exhibits the most intense band, a violet band at an R_f corresponding to the ursolic acid band in the chromatogram of *Standard solution A* and *Standard solution D*, and a greenish band at an R_f corresponding to the eugenol band or methyleugenol band in the chromatograms of *Standard solution B* or *Standard solution C*. [NOTE—Holy basil leaf may occur in two chemotypes, one characterized by the presence of eugenol and the other by the presence of methyleugenol.]

• D. HPLC

Analysis: Proceed as directed in the test for *Content of Triterpenes*.

Acceptance criteria: The chromatogram of the *Sample solution* exhibits two peaks at the retention times corresponding to the ursolic acid and oleanolic acid in the *Standard solutions*.

COMPOSITION

• CONTENT OF TRITERPENES

Mobile phase: A mixture of acetonitrile and a solution of 2.5 mg/mL ammonium acetate in water (67:33)

Standard solution A: 0.1 mg/mL of [USP Ursolic Acid RS](#) in methanol. Sonicate to dissolve if necessary.

Standard solution B: 4.0 mg/mL of [USP Powdered Holy Basil Extract RS](#) in methanol. Sonicate to dissolve if necessary. Before injection, pass through a membrane filter of 0.45-μm or finer pore size.

Sample solution: Transfer about 3.0 g of Powdered Holy Basil Leaf, accurately weighed, to a 100-mL round-bottom flask fitted with a reflux condenser. Add 50 mL of methanol, reflux on a water bath for 20 min, cool to room temperature, and decant the supernatant. Repeat until the last extract is colorless. Combine the extracts, filter, concentrate under vacuum and adjust the volume to 100 mL using methanol. Before injection, pass through a membrane filter having a 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, 205 nm

Column: 3.0-mm × 10-cm; 2.5-μm packing L1

Flow rate: 0.3 mL/min

Injection volume: 5 μL

System suitability

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

Suitability requirements

Chromatogram similarity: The chromatogram from *Standard solution B* is similar to the reference chromatogram provided with the lot of [USP Powdered Holy Basil Extract RS](#) being used.

Resolution: NLT 1.3 between the oleanolic acid and ursolic acid peaks, *Standard solution B*

Relative standard deviation: NMT 2% determined from the ursolic acid peak in repeated injections, *Standard solution A*

Analysis

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

Using the chromatograms of *Standard solution A*, *Standard solution B*, and the reference chromatogram provided with the lot of [USP Powdered Holy Basil Extract RS](#) being used, identify the retention time of the peaks corresponding to oleanolic acid and ursolic acid in the *Sample solution* chromatogram.

Calculate the percentage of triterpenes, oleanolic acid, and ursolic acid in the portion of Powdered Holy Basil Leaf taken:

$$\text{Result} = (r_u/r_s) \times C_s \times (V/W) \times 100$$

r_u = sum of peak areas of oleanolic acid and ursolic acid in the *Sample solution* chromatogram

r_s = peak area for ursolic acid in the *Standard solution A* chromatogram

C_s = concentration of ursolic acid in *Standard solution A* (mg/mL)

V = volume of the *Sample solution* (mL)

W = weight of Powdered Holy Basil Leaf taken to prepare the *Sample solution* (mg)