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Olive Leaf Dry Extract

DEFINITION

Olive Leaf Dry Extract is prepared from the dried leaf of *Olea europaea* L. (Fam. Oleaceae) by extraction with hydroalcoholic mixtures, ethyl acetate, or other suitable solvents. It contains NLT 90% and NMT 110% of the labeled amount of oleuropein ($C_{25}H_{32}O_{13}$), calculated on the dried basis. The ratio of starting plant material to extract is between 5:1 and 60:1. It may contain suitable added substances.

IDENTIFICATION

• A. [HPTLC FOR ARTICLES OF BOTANICAL ORIGIN \(203\)](#)

Flavonoids

Standard solution A: 1 mg/mL of [USP Rutin RS](#) and 2 mg/mL each of [USP Verbascoside RS](#), [USP Oleuropein RS](#), and [USP Oleanolic Acid RS](#) in methanol

Standard solution B: 25 mg/mL of [USP Olive Leaf Dry Extract RS](#) in methanol. Sonicate for 10 min, centrifuge, and use the supernatant.

Sample solution: Suspend about 125 mg of Olive Leaf Dry Extract in 5 mL of methanol, and sonicate for 10 min. Centrifuge, and use the supernatant.

Chromatographic system

Adsorbent: Chromatographic silica gel with an average particle size of 5 µm (HPTLC plate)¹

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

Relative humidity: Condition the plate to a relative humidity of 33%.

Temperature: Ambient temperature, not to exceed 30°

Developing solvent system: Ethyl formate, formic acid, toluene, and water (60:8:3:6)

Developing distance: 6 cm

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

Analysis

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

Apply the *Samples* as bands and dry in air. Develop in a saturated chamber and dry in air. Heat at 100° for 3 min. While the plate is still warm, treat with *Derivatization reagent A*, and examine under long-wave UV light (365 nm).

[NOTE—Immediately after examination, use the same developed and derivatized plate for *Identification B*.]

System suitability: Under long-wave UV (365 nm), the derivatized chromatogram of *Standard solution B* displays, in its lower third, three yellowish bands: the first close to the application line; the second located just above, or coincident with, the rutin band in *Standard solution A*; the third, due to luteolin-7-*O*-glucoside, positioned above the light-blue band corresponding to verbascoside in *Standard solution A*. There is a bluish-green band just above the band due to rutin. A faint light-blue band may appear just below the verbascoside band. The bands due to verbascoside and luteolin-7-*O*-glucoside are clearly separated. Above the yellow band due to luteolin-7-*O*-glucoside, there is a faint blue zone and a faint yellow band.

Acceptance criteria: Under long-wave UV light (365 nm), the chromatogram of the *Sample solution* displays the bands similar in position and color to those seen in *Standard solution B*. Additional faint bands may be observed.

• B. [HPTLC FOR ARTICLES OF BOTANICAL ORIGIN \(203\)](#)

Secoiridoids and Triterpenoids

[NOTE—Apply *Derivatization reagent B* to the plate used earlier in *Identification A*. Perform the second derivatization within 20 min of the first one.]

Chromatographic system

Derivatization reagent B: 85 mL of ice-cold methanol combined with 10 mL of glacial acetic acid, 5 mL of sulfuric acid, and 0.5 mL of *p*-anisaldehyde

Analysis

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

Treat the plate with *Derivatization reagent B*, dry in air, heat for 3 min at 100°, and immediately examine under white light.

System suitability: Under white light, the derivatized chromatogram of *Standard solution B* exhibits, in its lower third, a number of faint grayish-brown bands. In the middle of the chromatogram, an intense gray-brown band corresponding to oleuropein in *Standard solution A* appears. An intense violet band, corresponding to the oleanolic acid band in *Standard solution A*, is seen near the solvent front, and a weaker blue band due to maslinic acid just below it.

Acceptance criteria: Under white light, the chromatogram of the *Sample solution* displays the bands similar in position and color to those seen in *Standard solution B*; however, the bands due to oleanolic and maslinic acids may be faint or absent. Additional bands may be observed.

COMPOSITION

• CONTENT OF OLEUROPEIN

Mobile phase: 0.1% phosphoric acid in water and acetonitrile (80:20)

Standard solution: 0.2 mg/mL of [USP Oleuropein RS](#) in *Mobile phase*

Sample solution: Accurately weigh the amount of Olive Leaf Dry Extract calculated to contain 20 mg of oleuropein into a 50-mL volumetric flask, add 25 mL of *Mobile phase*, and sonicate for 5 min. Dilute with *Mobile phase* to volume, and mix well. Pass through a PTFE filter of 0.45-μm or finer pore size, discarding the initial few milliliters of the filtrate.

Chromatographic system

(See [Chromatography \(621\)](#), [System Suitability](#).)

Mode: HPLC

Detector: UV 230 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Column temperature: 25°

Flow rate: 1.5 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0 for the oleuropein peak

Relative standard deviation: NMT 2.0% determined for the oleuropein peak in replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Using the chromatogram of the *Standard solution*, identify the retention time of the peak corresponding to oleuropein in the *Sample solution*.

Calculate the percentage of oleuropein ($C_{25}H_{32}O_{13}$) in the portion of Olive Leaf Dry Extract taken:

$$P = (r_U/r_S) \times C_S \times (V/W) \times 100$$

r_U = peak area of oleuropein from the *Sample solution*

r_S = peak area of oleuropein from the *Standard solution*

C_S = concentration of [USP Oleuropein RS](#) in the *Standard solution* (mg/mL)

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

W = weight of Olive Leaf Dry Extract taken to prepare the *Sample solution* (mg)

Calculate the percentage of the labeled amount of oleuropein ($C_{25}H_{32}O_{13}$) in the portion of Olive Leaf Dry Extract taken:

$$\text{Result} = (P/L) \times 100$$

P = oleuropein content as determined above

L = labeled amount of oleuropein

Acceptance criteria: 90.0%–110.0% on the dried basis

CONTAMINANTS

Change to read:

- ▲ [BOTANICAL EXTRACTS \(565\)](#), [Preparations](#), [General Pharmacopeial Requirements](#), [Pesticide Residues](#): ▲ (CN 1-M-2020) Meets the requirements
- [BOTANICAL EXTRACTS \(565\)](#), [General Pharmacopeial Requirements](#), [Residual Solvents](#): Meets the requirements