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## Rosemary Leaf Dry Aqueous Extract

### DEFINITION

Rosemary Leaf Dry Aqueous Extract is prepared from Rosemary by extraction with water. It contains NLT 90% and NMT 110% of the labeled amount of rosmarinic acid, on the dried basis. It may contain suitable added substances as carriers.

### IDENTIFICATION

#### • A. THIN-LAYER CHROMATOGRAPHY

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

**Standard solution B:** 100 mg/mL of [USP Powdered Rosemary Hydrophilic Extract RS](#) in methanol

**Sample solution:** 100 mg/mL of Rosemary Leaf Dry Aqueous Extract in methanol

#### Chromatographic system

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

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

**Application volume:** 2 µL of the *Standard solution A*, and 4 µL of the *Standard solution B* and *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. Develop the chromatograms in a saturated chamber, remove the plate from the chamber, 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* shows, 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 band, right above the band due to rosmarinic acid, corresponding to caffeic acid. The bands due to rosmarinic acid and caffeic acid are clearly separated.

**Acceptance criteria:** The chromatogram of the *Sample solution* exhibits the following main bands similar in positions and colors to the corresponding bands in the chromatogram of *Standard solution B*. A blue fluorescent band at an  $R_f$  corresponding to the rosmarinic acid band in *Standard solution A*; a band at an  $R_f$  corresponding to the caffeic acid; a green band at about the middle of the chromatogram and an intense orange band in the lower third section of the chromatogram; below the green band, the *Sample solution* chromatogram exhibits a pattern of characteristically colored bands of low intensity (distinction from sage leaf, thyme leaf, holy basil leaf, basil leaf, and oregano leaf).

The *Sample solution* chromatogram does not show a pair of major orange bands in the lower-third section of the chromatogram (distinction from sage leaf and thyme leaf), a light blue band at an  $R_f$  of about one-third of the chromatogram (distinction from holy basil leaf), an orange band in the lower-third section of the chromatogram and a light blue band at about two-thirds of the chromatogram (distinction from basil leaf), or a red band right above the band due to caffeic acid in the upper-third section of the chromatogram (distinction from thyme leaf and oregano leaf). [NOTE—This red band is different from the red band, at the solvent front, present in Rosemary and other leaves due to chlorophyll.]

#### • B. HPLC

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

**Acceptance criteria:** The chromatogram of the *Sample solution* exhibits the major peak at the retention time corresponding to the rosmarinic acid peak in the chromatogram of *Standard solution A*. It also shows a less intense peak corresponding to the luteolin-3-O-glucuronide peak in the chromatogram of *Standard solution B* and at a relative retention time of about 0.8 compared to the rosmarinic acid peak.

**COMPOSITION****Change to read:**• **CONTENT OF ROSMARINIC ACID**

**Mobile phase:** A mixture of acetonitrile and a solution of 0.1% trifluoroacetic acid in water (32:68)

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

**Standard solution A:** 0.3 mg/mL of [USP Rosmarinic Acid RS](#) in *Solvent*. Sonicate to dissolve if necessary.

**Standard solution B:** 10 mg/mL of [USP Powdered Rosemary Hydrophilic Extract RS](#) in *Solvent*. Sonicate to dissolve if necessary. Before injection, pass through a membrane filter of  $\Delta 0.45\text{-}\mu\text{m}$ ▲ (ERR 1-May-2023) or finer pore size, discarding the first few mL of the filtrate.

**Sample solution:** Sonicate for 5 min an amount of Rosemary Leaf Dry Aqueous Extract equivalent to 15 mg of rosmarinic acid in 50 mL of *Solvent*. Before injection, pass through a membrane filter of  $\Delta 0.45\text{-}\mu\text{m}$ ▲ (ERR 1-May-2023) or finer pore size, discarding the first few mL of the filtrate.

**Chromatographic system**

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

**Mode:** LC

**Detector:** UV, 328 nm

**Column:** 4.6-mm × 25-cm; 5- $\mu\text{m}$  packing L1

**Column temperature:**  $20 \pm 1^\circ$

**Flow rate:** 1.0 mL/min

**Injection volume:** 5  $\mu\text{L}$

**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 Rosemary Hydrophilic Extract RS](#) being used.

**Tailing factor:** Between 0.90 and 1.30 for the rosmarinic acid peak, *Standard solution A*

**Relative standard deviation:** NMT 2% determined from the rosmarinic 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 Rosemary Hydrophilic Extract RS](#) being used, identify the retention time of the peak corresponding to rosmarinic acid in the *Sample solution* chromatogram.

Calculate the percentage of rosmarinic acid in the portion of Rosemary Leaf Dry Aqueous Extract taken:

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

$r_U$  = peak area for rosmarinic acid in the *Sample solution* chromatogram

$r_S$  = peak area for rosmarinic acid in *Standard solution A* chromatogram

$C_S$  = concentration of rosmarinic acid in *Standard solution A* (mg/mL)

$C_U$  = concentration of Dry Aqueous Extract in the *Sample solution* (mg/mL)

Calculate the percentage of the labeled amount of rosmarinic acid in the portion of Rosemary Leaf Dry Aqueous Extract taken:

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

$P$  = percentage of rosmarinic acid as determined above (%)

$L$  = labeled amount of rosmarinic acid (%)

**Acceptance criteria:** 90%–110% on the dried basis

**CONTAMINANTS**• **ELEMENTAL IMPURITIES—PROCEDURES (233)****Acceptance criteria**

**Arsenic:** NMT 1.0  $\mu\text{g/g}$

**Cadmium:** NMT 0.5  $\mu\text{g/g}$

**Lead:** NMT 5.0  $\mu\text{g/g}$