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Powdered Rosemary

DEFINITION

Powdered Rosemary is Rosemary reduced to powder or very fine powder. It contains NLT 2.0% of phenolic diterpenes, calculated as the sum of carnosic acid and carnosol, on the dried basis.

IDENTIFICATION

• **A.** Powdered Rosemary 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: 100 mg/mL of [USP Powdered Rosemary Hydrophilic Extract RS](#) in methanol

Sample solution: Sonicate for 10 min about 1 g of Powdered Rosemary in 10 mL of methanol. Centrifuge, and use supernatant.

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 *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 (see [Chromatography \(621\)](#)), 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* 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 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.]

• **C. HPLC**

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

Acceptance criteria: The chromatogram of the *Sample solution* exhibits the major peak at the retention time corresponding to carnosic acid in the chromatogram of *Standard solution A* and an additional peak corresponding to carnosol at a relative retention time of about 0.64 compared to that of the carnosic acid peak.

COMPOSITION**Change to read:**• **CONTENT OF PHENOLIC DITERPENES**

Mobile phase: A mixture of acetonitrile and a solution of 0.5% phosphoric acid in water (65:35)

[NOTE—Proceed under subdued light.]

Solvent: 0.5% phosphoric acid in methanol

Standard solution A: [NOTE—Use clear glassware.] 0.2 mg/mL of [USP Carnosic Acid RS](#) in *Solvent*. Sonicate to dissolve if necessary.

Sample solution: 1.0 g of Powdered Rosemary in 50 mL of methanol. Sonicate for 3 h. Before injection, pass through a membrane filter of

▲0.45-μm▲ (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, 230 nm

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

Column temperature: 25 ± 1°

Flow rate: 1.5 mL/min

Injection volume: 5 μL

System suitability

Sample: *Standard solution A*

Suitability requirements

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

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

Analysis

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

[NOTE—*Standard solution A* and *Sample solution* are stable for 12 h at room temperature.]

Using the chromatograms of *Standard solution A*, identify the retention time of the peaks corresponding to carnosic acid and carnosol in the *Sample solution* chromatogram. [NOTE—The approximate relative retention times for the carnosol and carnosic acid peaks are 0.64 and 1.00, respectively.]

Calculate the percentages of carnosic acid and carnosol in the portion of Powdered Rosemary taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times F \times 100$$

r_U = peak area of the relevant analyte in the *Sample solution* chromatogram

r_S = peak area for carnosic acid in the *Standard solution A* chromatogram

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

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

W = weight of Powdered Rosemary taken to prepare the *Sample solution* (mg)

F = conversion factor (1.00 for carnosic acid and 0.92 for carnosol)

Add the percentages of carnosic acid and carnosol.

Acceptance criteria: NLT 2.0% on the dried basis

CONTAMINANTS• [ELEMENTAL IMPURITIES—PROCEDURES \(233\)](#)**Acceptance criteria**

Arsenic: NMT 1.0 μg/g

Cadmium: NMT 0.5 μg/g

Lead: NMT 5.0 μg/g

Mercury: NMT 1.0 μg/g

• [ARTICLES OF BOTANICAL ORIGIN \(561\)](#), [Pesticide Residue Analysis](#): Meets the requirements

• [MICROBIAL ENUMERATION TESTS—NUTRITIONAL AND DIETARY SUPPLEMENTS \(2021\)](#): The total aerobic bacterial count does not exceed 10⁵ cfu/g, the total combined molds and yeasts count does not exceed 10³ cfu/g, and the bile-tolerant Gram-negative bacteria do not exceed 10² cfu/g