

## Rosemary Extract

Rosemary Extract is an extract obtained through extraction of the leaves or flowers of *Rosemarinus officinalis* LINNE (Lamiaceae), containing either carnosic acid and carnosol or rosmarinic acid as its primary ingredient, which is called Non-Water Soluble Rosemary Extract or Water-Soluble Rosemary Extract, respectively.

### Non-Water Soluble

**Content** Non-Water Soluble Rosemary Extract contains carnosic acid and carnosol in a combined amount not less than 10 %, and contains not less than 80% and not more than 120% of the labeled amount of carnosic acid and carnosol (content claimed by the manufacturer).

**Description** Non-Water Soluble Rosemary Extract occurs as a yellow-brown to brown powder, paste or liquid. It has a characteristic odor.

### Identification

- (1) Based on the labeled amount of Non-Water Soluble Rosemary Extract, take an amount of the extract corresponding to 10 mg amount, which is calculated on 10% of the combined carnosic acid and carnosol content basis, add 50 mL of water, and shake well: it is practically insoluble.
- (2) Based on the labeled amount of Non-Water Soluble Rosemary Extract, weigh an amount of the extract corresponding to 10 mg amount that is calculated on 10% of the combined carnosic acid and carnosol content basis, dissolve in 20 mL of a solution of phosphoric acid in methanol (0.5 → 100), filter through a membrane filter (0.45 µm in pore size), and use the filtrate as the test solution. Separately, weigh 1 mg of Carnosic Acid for Assay and 1 mg of Carnosol for Assay, dissolve each in 20 mL of the solution of phosphoric acid in methanol (0.5 → 100), filter each solution through a membrane filter (0.45 µm in pore size), and use these solutions as standard solution A and standard solution B, respectively. Perform the test with 10 µL each of the test solution and the standard solutions as directed under the Liquid Chromatography according to the following conditions: The retention times of the main peaks obtained from the test solution are the same as that of carnosic acid peak obtained from standard solution A and that of the carnosol peak obtained from standard solution B.

#### Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column : Octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column tube: A stainless steel tube 4 to 5 mm in inside diameter and 15 to 30 cm in length.

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of acetonitrile and 0.5 vol% aqueous phosphoric acid solution (65:35).

Flow rate: Adjust the flow rate so that the retention time of carnosic acid is 9 to 10 minutes.

### Purity

- (1) Lead—Not more than 2 µg/g as Pb (0.50 g, Method 1, Control solution, 4 mL of Standard Lead Solution, Flame method).
- (2) Arsenic—Not more than 3 µg/g as As (0.50 g, Method 3, Standard Color, 3.0 mL of Standard Arsenic Solution, Apparatus B).

**Assay** Based on the labeled amount of Non-Water Soluble Rosemary Extract, weigh accurately an amount of the extract corresponding to about 50 mg amount that is calculated on 10% of the combined carnosic acid and carnosol content basis, dissolve in a solution of phosphoric acid in methanol (0.5 → 100) to make exactly 100 mL, then filter through a membrane filter (0.45 µm in pore size), and use the filtrate as the test solution. Separately, weigh accurately about 10 mg of Carnosic Acid for Assay and about 10 mg of Carnosol for Assay, dissolve each in the solution of phosphoric acid in methanol (0.5 → 100) to make exactly 100 mL, filter each solution through a membrane filter (0.45 µm in pore size), and use these solution as standard solution A and standard solution B, respectively. Perform the test with 10 µL each of

the test solution and the standard solutions as directed under the Liquid Chromatography according to the following conditions. Determine the peak areas, CAu and CAs, of carnosic acid obtained from the test solution and standard solution A and the peak areas, CRu and CRs, of carnosol obtained from the test solution and standard solution B, and calculate the combined carnosic acid and carnosol content according to the following formula:

Combined carnosic acid and carnosol content (%) =  $\frac{CAu}{CAs} \times [\frac{CA}{\text{Concentration } (\mu\text{g/mL}) \text{ of the test solution}}] \times 100 + \frac{CRu}{CRs} \times [\frac{CR}{\text{Concentration } (\mu\text{g/mL}) \text{ of the test solution}}] \times 100$

, wherein

CA: Concentration ( $\mu\text{g/mL}$ ) of carnosic acid in standard solution A

CR: Concentration ( $\mu\text{g/mL}$ ) of carnosol in standard solution B

CAu: Peak area of carnosic acid obtained from the test solution

CAs: Peak area of carnosic acid obtained from standard solution A

CRu: Peak area of carnosol obtained from the test solution

CRs: Peak area of carnosol obtained from standard solution B

#### Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: Octadecylsilanized silica gel for liquid chromatography (5  $\mu\text{m}$  in particle diameter).

Column tube: A stainless steel tube 4 to 5 mm in inside diameter and 15 to 30 cm in length.

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of acetonitrile and 0.5 vol% aqueous phosphoric acid solution (65:35).

Flow rate: Adjust the flow rate so that the retention time of carnosic acid is 9 to 10 minutes.