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Garlic Delayed-Release Tablets

DEFINITION

Garlic Delayed-Release Tablets are prepared from Powdered Garlic or Powdered Garlic Extract and contain NLT 90.0% and NMT 140.0% of the labeled amount of alliin ($C_6H_{11}NO_3S$) and NLT 90.0% and NMT 140.0% of the labeled amount of potential allicin ($C_6H_{10}OS_2$).

IDENTIFICATION

• A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST

Standard solution A: 0.5 mg/mL of [USP L-Methionine RS](#)

Standard solution B: 0.5 mg/mL of [USP Alliin RS](#), in a mixture of methanol and water (1:1)

Sample solution: Transfer an amount of pulverized Tablets, equivalent to 30 mg of alliin, to a 100-mL volumetric flask. Add 70 mL of a mixture of methanol and water (1:1), shake, and centrifuge. Concentrate to a small volume (about 5 mL) using a rotary evaporator.

Chromatographic system

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

Application volume: 20 μ L, applied separately as 10-mm bands

Developing solvent system: Butyl alcohol, *n*-propyl alcohol, glacial acetic acid, and water (3:1:1:1)

Spray reagent: 2 mg/mL of ninhydrin, in a mixture of butyl alcohol and 2 N acetic acid (19:1)

Analysis

Samples: *Standard solutions* and *Sample solution*

Proceed as directed in the chapter. Spray with the *Spray reagent*, heat at 100°–105° for 10 min, and immediately examine the plate.

Acceptance criteria: The chromatogram of the *Sample solution* shows the following orange and pinkish violet zones: a violet zone having an R_F value of 0.89; a pink zone having an R_F value of 0.5 and corresponding in color and R_F value to that of the chromatogram of *Standard solution A*; a pinkish zone having an R_F value of 0.43; a strong orange zone having an R_F value of 0.38; a pinkish violet zone having an R_F value of 0.3 and corresponding in color and R_F value to that of the chromatogram of *Standard solution B*; and additional pinkish orange zones situated very close to each other, just below the zone attributed to alliin in the chromatogram of *Standard solution B*.

• B. HPLC IDENTIFICATION TEST

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

Acceptance criteria: The *Sample solution* exhibits a peak for alliin corresponding to one of the diastereoisomer pairs of peaks in the *Standard solution*.

STRENGTH

• CONTENT OF ALLIIN

Alliinase inhibitor solution: Dissolve 109 mg of carboxymethoxylamine hemihydrochloride in 100.0 mL of water.

Solution A: Dissolve 1.24 g of monobasic sodium phosphate in 100 mL of water, adjust with 0.2 M sodium hydroxide to a pH of 7.1, and dilute with water to 200.0 mL.

Buffer: Dissolve 1.38 g of monobasic sodium phosphate in 100 mL of water, adjust with 0.2 M sodium hydroxide to a pH of 9.5, and dilute with water to 200.0 mL.

Derivatization reagent: Dissolve 140 mg of *o*-phthaldialdehyde in 5 mL of methanol, add 100 μ L of *t*-butylthiol, and dilute with *Buffer* to 50 mL.

[NOTE—This reagent may occasionally become opaque during preparation. Store at room temperature, and use within 1 week.]

Mobile phase: Acetonitrile, 1,4-dioxane, tetrahydrofuran, and *Solution A* (25:2.9:2.2:69.9)

Standard solution: 0.05 mg/mL of [USP Alliin RS](#) in a mixture of methanol and water (1:1). Use a syringe to transfer 0.1 mL of this solution to a septum-capped vial, and add 0.5 mL of the *Derivatization reagent*. Allow a reaction time of NLT 2 min before injection into the chromatograph.

Sample solution: Pulverize a counted number of Tablets, equivalent to 50 mg of alliin, with a mortar and pestle. Transfer a quantity of powder equivalent to 5 mg of alliin to a 100-mL volumetric flask. Add 70 mL of *Alliinase inhibitor solution*, and shake for 1 min. Dilute with *Alliinase*

inhibitor solution to volume. Use a volumetric syringe to transfer 0.1 mL of this solution to a septum-capped vial, and add 0.5 mL of the *Derivatization reagent*. Allow a reaction time of NLT 2 min before injection into the chromatograph.

Chromatographic system

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

Mode: LC

Detector: UV 337 nm

Column: 4-mm × 10-cm; packing L1

Flow rate: 1 mL/min

[NOTE—Alliin exhibits two major peaks, representing its diastereomers.]

Injection size: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0% for each of the major peaks

Analysis

Samples: *Standard solution* and *Sample solution*

[NOTE—Record the chromatograms, and measure the areas of the responses of the alliin diastereomer peaks.]

Calculate the percentage of alliin in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area for alliin from the *Sample solution*

r_S = sum of the peak area for alliin diastereomers from the *Standard solution*

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

C_U = nominal concentration of alliin in the *Sample solution* (µg/mL)

Acceptance criteria: 90%–140.0%

• CONTENT OF POTENTIAL ALLICIN

Alliinase inhibitor solution: Dissolve 109 mg of carboxymethoxylamine hemihydrochloride in 100.0 mL of water.

Crude alliinase solution: Homogenize 5 g of raw garlic cloves with 25 mL of water. Filter, and extract three times with 50 mL of *tert*-butyl methyl ether. Discard the organic phase, and remove the residual solvent from the aqueous phase by rotary evaporation in vacuum for 5 min. Filter, and store frozen in small vials. [NOTE—This solution is stable for 6 months when stored as directed. Thaw at room temperature just before use.]

Mobile phase: Methanol and water (3:2)

Standard stock solution: 50 µg/mL of [USP Alliin RS](#)

Standard solution: Transfer 1.0 mL of the *Standard stock solution* to a 5-mL volumetric flask containing 100 µL of *Crude alliinase solution*, and allow to stand for 5 min at room temperature. Dilute with water to volume, and pass through a filter having a 0.45-µm or finer pore size.

Sample solution: Transfer an equivalent to 5 mg of potential alliin, from finely powdered Tablets (NLT 20), to a 200-mL volumetric flask, and add 25 mL of water. Incubate at room temperature for exactly 30 min. Stop the enzymatic reaction by diluting with *Alliinase inhibitor solution* to volume. Centrifuge a portion of this solution, transfer 1.0 mL of the supernatant to a 5-mL volumetric flask, and dilute with water to volume.

Blank solution: 100 µL of *Crude alliinase solution* diluted with water to 1 mL

Chromatographic system

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

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 1 mL/min

Injection size: 100 µL

System suitability

Samples: *Standard solution*, *Sample solution*, and *Blank solution*

[NOTE—The alliin peak is identified by comparing the chromatograms of the *Blank solution* and the *Standard solution*.]

Suitability requirements