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Powdered Garlic

DEFINITION

Powdered Garlic is produced from Garlic that has been cut, freeze-dried or dried at a temperature not exceeding 65°, and powdered. It contains NLT 0.3% of alliin and NLT 0.1% of γ -glutamyl-(S)-allyl-L-cysteine, calculated on the dried basis.

IDENTIFICATION

• A. THIN-LAYER CHROMATOGRAPHY

Standard solution A: 0.5 mg/mL of [USP L-Methionine RS](#) in a mixture of methanol and water (1:1)

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

Sample solution: Transfer 1 g of the Powdered Garlic to an extractor, and extract with two 20-mL portions of a mixture of methanol and water (1:1), combining the extracts. Concentrate to a small volume (about 5 mL), using a rotary evaporator.

Chromatographic system

Adsorbent: 0.25-mm layer of chromatographic silica gel, typically 20 cm long (TLC plates)

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)

Derivatization reagent: 0.2 in 100 solution of ninhydrin in a mixture of butyl alcohol and 2 N acetic acid (19:1)

Analysis

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

Develop the chromatograms until the solvent front has moved up about three-fourths of the plate, in a saturated chamber. Remove the plate, and allow the solvent to evaporate. Spray with *Derivatization reagent*, heat at 100°–105° for 10 min, and immediately examine the plate under white light.

Acceptance criteria: The chromatogram of the *Sample solution* shows the following zones: a violet zone having an R_f value of about 0.89; a pink zone having an R_f value of about 0.5 and corresponding in color and R_f value to that obtained from the chromatogram of *Standard solution A*; a pinkish zone having an R_f value of about 0.43; a strong orange zone having an R_f value of about 0.38; a pinkish violet zone having an R_f value of about 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 corresponding to alliin in the chromatogram of *Standard solution B*.

• B.

Sample: About 10 g of Powdered Garlic

Analysis: Transfer to a suitable flask. Add 10 mL of 1 N sodium hydroxide and 10 mL of water, heat the flask in boiling water for 10 min, cool, and filter. Add a few drops of freshly prepared sodium nitroferricyanide TS to 2 mL of the filtrate.

Acceptance criteria: The appearance of a red or orange-red color indicates the presence of sulfur-containing compounds in the *Sample*.

• C. The retention time of the major peak in the *Sample solution* corresponds to that of one of the alliin diastereomer peaks in the *Standard solution*, as obtained in the test for *Content of Alliin*.

• D. THIN-LAYER CHROMATOGRAPHY

Extraction column: 1-cm $\times$ 5-cm solid-phase extraction column containing styrene-divinylbenzene copolymer packing with a 75- to 150- μ m diameter and a 400- to 600-Å pore size. Condition column before use by washing with 50 mL of methanol and with 50 mL of a mixture of methanol and water (3:7). [NOTE—Do not allow the column to dry.]

Standard solution: 0.2 mg/mL each of USP β -Chlorogenin RS and [USP Agigenin RS](#) in methanol

Sample solution: Transfer about 10 g of Powdered Garlic to a 37-mL homogenizing cup, and homogenize with 25 mL of methanol at the highest speed for 1 min. Centrifuge the mixture, and decant the supernatant to a flask. Add 70 mL of water. Transfer to the *Extraction column*, allow to drain, and discard the eluate. Wash the column with 50 mL of a mixture of methanol and water (3:7), allow the solvent mixture to drain, and discard the eluate. Finally, elute the crude saponin fraction off the column with 20 mL of methanol, collect the eluate, and evaporate to dryness. Dissolve the residue in 4 mL of a mixture of 8% sulfuric acid and alcohol (1:1), transfer the solution to a screw-capped test tube, and heat on a boiling water bath for 5 h. Cool the test tube, add 20 mL of water, and transfer the solution to a freshly conditioned *Extraction column*, allow to drain, and discard the eluate. Wash the column with 30 mL of a mixture of methanol and water (7:3), and discard the eluate. Finally, elute the column with 50 mL of methanol. Collect the eluate, evaporate it to dryness, and dissolve the residue in 0.5 mL of methanol.

Chromatographic system

Adsorbent: 0.25-mm layer of chromatographic silica gel, typically 20 cm long (TLC plates)

Application volume: 20 μ L, as 7-mm bands

Developing solvent system: Methylene chloride and methanol (15:2)

Derivatization reagent: Dissolve 0.5 mL of 4-methoxybenzaldehyde and 0.5 mL of sulfuric acid in sufficient alcohol to make 10 mL.

Analysis

Samples: *Standard solution* and *Sample solution*

Develop the chromatograms until the solvent front has moved up about three-fourths of the plate, in a saturated chamber. Remove the plate, and allow the solvent to evaporate. Spray the plate with *Derivatization reagent*, heat the plate at 100°–105° for 5 min, and examine the plate under white light.

Acceptance criteria: The chromatogram of the *Sample solution* exhibits, among several yellowish and grayish green spots, a grayish green spot at an R_f value of about 0.4, corresponding to the grayish green spot due to β -chlorogenin of the *Standard solution*. The chromatogram of the *Sample solution* does not exhibit a spot at an R_f value of about 0.2, corresponding to agigenin of the *Standard solution*.

COMPOSITION

• CONTENT OF ALLIIN

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

Solution A: 0.045 M monobasic sodium phosphate in water. Adjust with 0.2 M sodium hydroxide to a pH of 7.1.

Buffer: 0.05 M monobasic sodium phosphate in water. Adjust with 0.2 M sodium hydroxide to a pH of 9.5.

Derivatization reagent: Dissolve 140 mg of o-phthalaldehyde 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)

Sample stock solution: Transfer about 1.0 g of Powdered Garlic, accurately weighed, to a flask. Add 30.0 mL of *Alliinase inhibitor solution*, and shake vigorously until the powder is fully dispersed. Centrifuge to obtain a clear solution.

Sample solution: Transfer 5.0 mL of the *Sample stock solution* to a 10-mL volumetric flask, and dilute with *Alliinase inhibitor solution* to volume.

Chromatographic system

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

Mode: LC

Detector: UV 337 nm

Column: 4-mm $\times$ 10-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 10 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

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

Relative standard deviation: NMT 2.0% for each of the major peaks, in repeated injections

Analysis

Samples: *Standard solution* and *Sample solution*

Using a syringe, transfer 0.1 mL of the *Standard solution* or the *Sample solution* to separate septum-capped vials, add 0.5 mL of the *Derivatization reagent* to each vial, and mix. Allow a reaction time of NLT 2 min before injection into the chromatograph. Record the chromatograms, and measure the areas of the alliin diastereomer peaks.

Calculate the percentage of alliin in the portion of Powdered Garlic taken:

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

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

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

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

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

W = weight of Powdered Garlic used to prepare the *Sample stock solution* (mg)

D = dilution factor to prepare the *Sample solution* from the *Sample stock solution*, 2

Acceptance criteria: NLT 0.3% on the dried basis

• CONTENT OF γ -GLUTAMYL-(S)-ALLYL-L-CYSTEINE

Solution A: Dissolve 6.80 g of monobasic potassium phosphate in 900 mL of water, and adjust with phosphoric acid to a pH of 2.6. Dilute with water to 1000.0 mL, and mix.

Mobile phase: Methanol and *Solution A* (3:17)

Standard solution: 0.08 mg/mL of USP γ -Glutamyl-(S)-allyl-L-cysteine RS in a mixture of methanol and water (1:1)