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# Garlic

## DEFINITION

Garlic consists of the fresh or dried compound bulbs of *Allium sativum* L. (Fam. Liliaceae). It contains NLT 0.5% of alliin and NLT 0.2% of  $\gamma$ -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:** Cut a freeze-dried garlic bulb into small pieces, transfer 1 g of the cut pieces 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  $\mu$ 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% 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 the *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 garlic bulbs that have been cut into small pieces

**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- $\mu$ 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  $\beta$ -Chlorogenin RS and [USP Agigenin RS](#) in methanol

**Sample solution:** Transfer about 10 g of freshly peeled garlic cloves 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 µL, 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

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

**Solution A:** Monobasic sodium phosphate 0.045 M in water, adjusted with 0.2 M sodium hydroxide to a pH of 7.1

**Buffer:** Monobasic sodium phosphate 0.05 M in water, adjusted with 0.2 M sodium hydroxide to a pH of 9.5

**Derivatization reagent:** Dissolve 140 mg of o-phthaldialdehyde in 5 mL of methanol, add 100 µL 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 10.0 g of freshly peeled garlic cloves, accurately weighed, to a 110-mL homogenizing cup. Add 70.0 mL of *Allinase inhibitor solution*, and blend at the highest speed for 30 s. Centrifuge, and decant the supernatant into a 100-mL volumetric flask. Mix the remaining solids in the cup with 20 mL of *Allinase inhibitor solution*, centrifuge, and add the supernatant to the volumetric flask. Dilute the contents of the flask with *Allinase inhibitor solution* to volume.

**Sample solution:** Dilute a portion of the *Sample stock solution* 1 in 10 with a mixture of methanol and water (1:1).

#### 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

**Injection volume:** 10 µL

#### System suitability

**Sample:** *Standard solution*

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

#### Suitability requirements

**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 *Sample solution* to separate septum-capped vials, and add 0.5 mL of the *Derivatization reagent* to each vial. 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 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*

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