

Status: Currently Official on 17-Feb-2025
 Official Date: Official as of 01-Jan-2018
 Document Type: Dietary Supplement Monographs
 DocId: GUID-B3467C8F-3C55-4ADF-9220-35C3437BA9C6_3_en-US
 DOI: https://doi.org/10.31003/USPNF_M34687_03_01
 DOI Ref: qor3k

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Powdered Garlic Extract

DEFINITION

Powdered Garlic Extract is prepared from fresh Garlic bulbs by extraction with alcohol. The ratio of the starting crude plant material to Powdered Extract is 9.5:1–13.5:1. It contains NLT 4.0% of alliin ($C_6H_{11}NO_3S$). It may contain added Powdered Garlic or other suitable substances.

IDENTIFICATION

• A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST

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 a quantity of Powdered Extract, equivalent to about 5 mg of alliin, to a suitable container. Add 40 mL of a mixture of methanol and water (1:1), and shake until the powder is fully dispersed. Centrifuge, and decant the supernatant into a round-bottomed flask. Concentrate to a small volume (about 5 mL) using a rotary evaporator.

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

Application volume: 20 µL, 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: 0.2% solution of ninhydrin in a mixture of butyl alcohol and 2 N acetic acid (19:1)

Analysis

Samples: *Standard solutions* 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 *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 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 of 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 attributed to alliin in the chromatogram of *Standard solution B*.

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

COMPOSITION

• CONTENT OF ALLIIN

Allinase inhibitor solution: 1.09 mg/mL of carboxymethoxylamine hemihydrochloride

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*-phthalaldehyde in 5 mL of methanol. Add 100 µL of *t*-butyl thiol, dilute with *Buffer* to 50 mL, and mix. [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 solution: Transfer about 0.10 g of Powdered Extract, accurately weighed, to a 50-mL volumetric flask. Add 30 mL of *Allinase inhibitor solution*, and shake until the Powdered Extract is fully dispersed. Dilute with *Allinase inhibitor solution* to volume. Centrifuge, transfer 5 mL of the clear supernatant to a 10-mL volumetric flask, and dilute with *Allinase inhibitor solution* to volume.

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 size: 10 µL

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

Analysis

Samples: *Standard solution* and *Sample solution*

Using a volumetric syringe, transfer 0.1 mL of the *Sample solution* or the *Standard 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 Powdered Extract taken:

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

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

r_S = 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 solution* (mL)

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

Acceptance criteria: NLT 4.0% on the dried basis

CONTAMINANTS

- [MICROBIAL ENUMERATION TESTS \(2021\)](#): The total aerobic bacterial count does not exceed 10^4 cfu/g, and the total combined molds and yeasts count does not exceed 10^3 cfu/g.
- [ABSENCE OF SPECIFIED MICROORGANISMS \(2022\)](#): Meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*

SPECIFIC TESTS

• ALLIINASE ACTIVITY

Alliinase inhibitor solution, Solution A, Buffer, Derivatization reagent, Mobile phase, Standard solution, Chromatographic system, and

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

Sample solution: Incubate 200 mg of Powdered Extract with 20 mL of water at room temperature for 5 min. Immediately after incubation, add 80.0 mL of *Alliinase inhibitor solution*, mix, and centrifuge.

Acceptance criteria: The area of the alliin peak of the *Sample solution* is NMT 1% of the area of the alliin peak of the *Standard solution*.

- [ALCOHOL DETERMINATION, Method III\(611\)](#): NMT 0.5%
- [ARTICLES OF BOTANICAL ORIGIN, Water Content\(561\)](#): NMT 5.0%
- **OTHER REQUIREMENTS:** Meets the requirements under [Botanical Extracts \(565\)](#), [Packaging and Storage and Pesticide Residues](#)

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, in a cool place, protected from light.
- **LABELING:** The label states the Latin binomial and, following the official name, the part of the plant from which the article was prepared. The label also indicates the content of alliin, the extracting solvent or solvent mixture used for preparation, and the ratio of the starting crude plant material to Powdered Extract. It meets the requirements under [Botanical Extracts \(565\)](#), [Labeling](#).
- [USP REFERENCE STANDARDS \(11\)](#).
[USP Alliin RS](#)
[USP L-Methionine RS](#)

Auxiliary Information - Please [check for your question in the FAQs](#) before contacting USP.

Topic/Question	Contact	Expert Committee
POWDERED GARLIC EXTRACT	Anton Bzhelyansky Scientific Liaison	BDSHM2020 Botanical Dietary Supplements and Herbal Medicines
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	BDSHM2020 Botanical Dietary Supplements and Herbal Medicines

Chromatographic Database Information: [Chromatographic Database](#)

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