Penicillamine Capsules

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Expert Committee	Chemical Medicines Monographs 1
Reason for Revision	Compliance

In accordance with the Rules and Procedures of the 2015–2020 Council of Experts, the Chemical Medicines Monographs 1 Expert Committee has revised the Penicillamine Capsules monograph. The purpose for the revision is to delete the *Loss on Drying* test, which is formulation specific.

The Penicillamine Capsules Revision Bulletin supersedes the currently official monograph.

Should you have any questions, please contact Christine Hiemer, Scientific Liaison (301-230-6351 or cwh@usp.org).

Penicillamine Capsules

DEFINITION

Penicillamine Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of penicillamine ($C_5H_{11}NO_2S$).

IDENTIFICATION

• **A. Thin-Layer Chromatography**

Standard solution: 100 mg of USP Penicillamine RS in 10 mL of methanol. Add 2 drops of 3 N hydrochloric acid and mix.

Sample solution: Transfer a portion of Capsule contents, containing nominally about 100 mg of penicillamine, to a 10-mL volumetric flask, and dilute with methanol to volume. Add 2 drops of 3 N hydrochloric acid, mix and filter. Use the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩*, General Procedures, Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture, heated at 105° for 30 min, and allowed to cool before use

Application volume: 10 µL

Developing solvent system: Butyl alcohol, glacial acetic acid, and water (8:2:2)

Spray reagent: 3-mg/mL solution of ninhydrin in dehydrated alcohol

Analysis

Samples: *Standard solution* and *Sample solution*

Separately apply the *Sample solution* and the *Standard solution* to the plate. Develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths the length of the plate. Remove the plate, mark the solvent front, allow the solvent to evaporate, and place the plate in an atmosphere of iodine vapors. After a few minutes, spray the plate with *Spray reagent*, heat it at 105° for 10 min, allow it to cool, and examine it.

Acceptance criteria: The R_F values, colors, and intensities of the principal spots from the *Sample solution* correspond to those from the *Standard solution*.

• **B. Procedure**

Solution A: 100 mg/mL of phosphotungstic acid in water

Sample solution: Dissolve a portion of Capsule contents, containing nominally about 20 mg of penicillamine, in 4 mL of water.

Analysis: To the *Sample solution*, add 2 mL of *Solution A* and heat nearly to boiling.

Acceptance criteria: A deep blue color is produced immediately.

ASSAY

• **Procedure**

Mobile phase: 6.9 g/L of monobasic sodium phosphate and 0.2 g/L of sodium 1-hexanesulfonate in water. Adjust with phosphoric acid to a pH of 3.0 ± 0.1.

Diluent: 1.0 g/L of edetate disodium in water

System suitability solution: 1 mg/mL of USP Penicillamine RS and 0.1 mg/mL of USP Penicillamine Disulfide RS in *Diluent*

Standard solution: 1.25 mg/mL of USP Penicillamine RS in *Diluent*

Sample solution: Nominally equivalent to 1.25 mg/mL of penicillamine in *Diluent* prepared as follows. Transfer the contents of NLT 10 Capsules to a suitable volumetric flask. Add the empty Capsule shells to the flask, and add sufficient *Diluent* to the flask to fill it to three-fourths of its capacity. Shake for 1 min, and allow the mixture to stand for 90 min. Dilute with *Diluent* to volume. Pass a portion of this solution through a suitable filter of 1-µm or finer porosity, and use the clear filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1.6 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for penicillamine and penicillamine disulfide are 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between penicillamine and penicillamine disulfide, *System suitability solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of penicillamine ($C_5H_{11}NO_2S$) in portion of Capsules taken:

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

r_U = peak response of penicillamine from the *Sample solution*

r_S = peak response of penicillamine from the *Standard solution*

C_S = concentration of USP Penicillamine RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of penicillamine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **Dissolution** ⟨711⟩

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 1: 100 rpm

Time: 30 min

Procedure for a pooled sample

Dilute hydrochloric acid: Dilute 37 mL of hydrochloric acid with water to 1 L.

Dilute sulfuric acid: Dilute 1 mL of sulfuric acid with water to 50 mL.

Ammonium sulfamate reagent: 2.5 mg/mL of ammonium sulfamate in *Dilute hydrochloric acid*

***N*-(1-Naphthyl)ethylenediamine dihydrochloride reagent:** 1 mg/mL of *N*-(1-naphthyl)ethylenediamine dihydrochloride in *Dilute hydrochloric acid*

Sulfanilamide–mercuric chloride reagent: 1 mg/mL of sulfanilamide and 1 mg/mL of mercuric chloride in *Dilute hydrochloric acid*

Sodium nitrite reagent: 2 mg/mL of sodium nitrite in *Dilute sulfuric acid*. Prepare fresh.

Standard solution: 250 µg/mL of USP Penicillamine RS in 0.1 N hydrochloric acid

Sample solution: Withdraw a portion of the solution under test, containing nominally about 278 µg of penicillamine, and pass through a suitable filter.

Blank: Volume of 0.1 N hydrochloric acid equivalent to a volume of the *Sample solution*

Instrumental conditions

Mode: UV-Vis

Analytical wavelength: 540 nm

Cell: 1 cm

Analysis: Pipet the *Sample solution* into a 100-mL volumetric flask. Into a similar flask, transfer the reagent *Blank*, and into a third 100-mL volumetric flask, pipet 1 mL of *Standard solution*. Treat each flask as follows. Add by pipet 3 mL of *Sodium nitrite reagent*, and mix by swirling occasionally. After 5 min, add 10 mL of *Ammonium sulfamate reagent*, swirl, and allow to stand for an additional 5 min. Add 5 mL of *Sulfanilamide–mercuric chloride reagent*, swirl, and immediately add 10 mL of *N-(1-Naphthyl)ethylenediamine dihydrochloride reagent*. Dilute with water to volume and mix. Determine the absorbances of both solutions against the *Blank*.

Calculate the percentage of labeled amount of penicillamine ($C_5H_{11}NO_2S$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times V \times (1/L) \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*
C_S = concentration of USP Penicillamine RS in the *Standard solution* (µg/mL)
C_U = nominal concentration of penicillamine in the *Sample solution* (µg/mL)
V = volume of the Medium, 900 mL
L = label claim (mg/Capsule)

Tolerances: NLT 80% (Q) of the labeled amount of penicillamine ($C_5H_{11}NO_2S$) is dissolved.

Procedure for a unit sample

Buffer solution: 50 mM solution of monobasic potassium phosphate buffer, pH 3.0

Mobile phase: Methanol and *Buffer solution* (3:97)

System suitability solution: 0.002 mg/mL of USP Penicillamine Disulfide RS in 0.1 N hydrochloric acid

Sample solution: Proceed as directed in *Dissolution* ⟨711⟩, *Procedure*. After 30 min, withdraw 10 mL of solution from each vessel, and immediately pass each aliquot through a 0.45-µm polyvinylidene difluoride filter paper. Discard the first 2 mL of filtered solution, and chromatograph the remaining filtrate.

Standard solution: USP Penicillamine RS in 0.1 N hydrochloric acid at a concentration similar to *Sample solution*.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1.0 mL/min

Injection volume: 30 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

Suitability requirements

Resolution: NLT 2.0 between penicillamine and penicillamine disulfide, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of penicillamine ($C_5H_{11}NO_2S$) released:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times V \times (1/L) \times 100$$

r_U = peak area from the *Sample solution*
r_S = peak area from the *Standard solution*
C_S = concentration of USP Penicillamine RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of in the *Sample solution* (mg/mL)
V = volume of Medium, 900 mL
L = label claim (mg/Capsule)

Tolerances: NLT 80% (Q) of the labeled amount of penicillamine ($C_5H_{11}NO_2S$) is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

• **Limit of Penicillamine Disulfide**

Mobile phase, Diluent, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution: 0.025 mg/mL of USP Penicillamine Disulfide RS in *Diluent*.

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for penicillamine and penicillamine disulfide are 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between penicillamine and penicillamine disulfide, *System suitability solution*

Relative standard deviation: NMT 2.0% for penicillamine disulfide, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of penicillamine disulfide ($C_{10}H_{20}N_2O_4S_2$) in the portion of Capsules taken:

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

r_U = peak area of penicillamine disulfide from the *Sample solution*
r_S = peak area of penicillamine disulfide from the *Standard solution*
C_S = concentration of USP Penicillamine Disulfide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of penicillamine in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 2.0%

SPECIFIC TESTS

Delete the following:

▲• **Loss on Drying** ⟨731⟩

Sample: 100 mg of Capsule contents.

Analysis: Dry *Sample* in a capillary-stoppered bottle in a vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 h.

Acceptance criteria: it loses NMT 1.0% of its weight.▲ (RB 11-Jun-2019)

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers.

• **USP Reference Standards** ⟨11⟩

USP Penicillamine RS

USP Penicillamine Disulfide RS

$C_{10}H_{20}N_2O_4S_2$