Penicillamine Capsules

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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 (C₅H₁₁NO₃S).

**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°C 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°C for 10 min, allow it to cool, and examine it.

  **Acceptance criteria:** The Rₜ 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 TLC solution

**Analysis**

**Samples:** Standard solution and Sample solution

Calculate the percentage of penicillamine (C₅H₁₁NO₃S) in portion of Capsules taken:

\[ \text{Result} = \left( \frac{r_d}{r_s} \right) \times \left( \frac{C_d}{C_s} \right) \times 100 \]

\[ r_d = \text{peak response of penicillamine from the Sample solution} \]

\[ r_s = \text{peak response of penicillamine from the Standard solution} \]

\[ C_d = \text{concentration of USP Penicillamine RS in the Standard solution (mg/mL)} \]

\[ C_s = \text{nominal concentration of penicillamine in the Sample solution (mg/mL)} \]

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS**

- **DISSOLUTION**

  **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

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**2 Penicillamine**

**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$_{13}$H$_{18}$N$_{2}$O$_{5}$S) dissolved:

\[
\text{Result} = \left( \frac{A_r}{A_s} \right) \times \left( \frac{C_s}{C_r} \right) \times V \times \left( \frac{1}{L} \right) \times 100
\]

- $A_r$ = 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)
- $C_r$ = 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$_{13}$H$_{18}$N$_{2}$O$_{5}$S) is dissolved.

**Procedure for a unit sample**

**Buffer solution:** 50 mL 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 **Dilution (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$_{13}$H$_{18}$N$_{2}$O$_{5}$S) released:

\[
\text{Result} = \left( \frac{r_r}{r_s} \right) \times \left( \frac{C_s}{C_r} \right) \times V \times \left( \frac{1}{L} \right) \times 100
\]

- $r_r$ = 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)
- $C_r$ = 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$_{13}$H$_{18}$N$_{2}$O$_{5}$S) 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 **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$_{26}$N$_{2}$O$_{8}$S$_{2}$) in the portion of Capsules taken:

\[
\text{Result} = \left( \frac{r_r}{r_s} \right) \times \left( \frac{C_s}{C_r} \right) \times 100
\]

- $r_r$ = 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)
- $C_r$ = nominal concentration of penicillamine in the Sample solution (mg/mL)

**Acceptance criteria:** NMT 2.0%

**SPECIFIC TESTS**

**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.

**ADDITIONAL REQUIREMENTS**

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

- **USP Reference Standards** (11)

  - USP Penicillamine RS
  - USP Penicillamine Disulfide RS

C$_{10}$H$_{26}$N$_{2}$O$_{8}$S$_{2}$

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