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Penicillamine Capsules

To view the Notice from the Expert Committee that posted in conjunction with this accelerated revision, please click www.uspnf.com/rb-penicillamine-caps-20230728.

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.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

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 Capsules (NLT 10) 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 pore size, and use the clear filtrate.

Chromatographic system

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

Mode: LC

Detector: UV 210 nm

Column: 3.9-mm × 30-cm; 10-µm 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 the 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

Change to read:

• **DISSOLUTION** ⟨711⟩

▲Test 1▲ (RB 1-Aug-2023)

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

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

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* (mg/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 at a pH of 3.0

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

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 polyvinylidene difluoride filter of 0.45-µm pore size. Discard the first 2 mL of filtered solution, and chromatograph the remaining filtrate.

System suitability solution: USP Penicillamine RS at a concentration similar to the *Sample solution* and 0.002 mg/mL of USP Penicillamine Disulfide RS in 0.1 N hydrochloric acid

Standard solution: USP Penicillamine RS in 0.1 N hydrochloric acid at a concentration similar to the *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: *System suitability solution* and *Standard 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: *Sample solution* and *Standard solution*

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

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

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Penicillamine RS in the *Standard 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.

▲**Test 2:** If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

Medium: 0.1 N hydrochloric acid; 500 mL

Apparatus 1: 100 rpm

Time: 30 min

Solution A: Transfer 10 mL of phosphoric acid to a 100-mL volumetric flask, and dilute with water to volume.

Buffer: Dissolve 6.8 g of potassium phosphate, monobasic in 1 L of water. Adjust with *Solution A* to a pH of 3.0.

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

Impurity stock solution: 0.1 mg/mL of USP Penicillamine Disulfide RS in *Medium*. Sonicate to dissolve.

System suitability solution: 0.5 mg/mL of USP Penicillamine RS and 0.01 mg/mL of USP Penicillamine Disulfide RS prepared as follows. Transfer an appropriate quantity of USP Penicillamine RS to a suitable volumetric flask. Add 10% of the flask volume of *Impurity stock solution*. Dilute with *Medium* to volume.

Standard solution: 0.5 mg/mL of USP Penicillamine RS in *Medium*. Sonicate to dissolve.

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.

Chromatographic system

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

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 15-cm; 3-µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

Column temperature: 30°

Run time: NLT 3 times the retention time of penicillamine

System suitability

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

[NOTE—The relative retention times for penicillamine and penicillamine disulfide are about 1.0 and 1.1, respectively.]

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

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

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)

V = volume of *Medium*, 500 mL

L = label claim (mg/Capsule)

Tolerances: NLT 80% (Q) of the labeled amount of penicillamine ($C_5H_{11}NO_2S$) is dissolved. ▲ (RB 1-Aug-2023)

• **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%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers. Store at controlled room temperature.

Add the following:

▲• **Labeling:** When more than one dissolution test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.▲ (RB 1-Aug-2023)

• **USP Reference Standards** ⟨11⟩

USP Penicillamine RS

USP Penicillamine Disulfide RS

3,3'-Dithiodi-D-valine.

$C_{10}H_{20}N_2O_4S_2$ 296.40

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
PENICILLAMINE CAPSULES	Documentary Standards Support	SM12020 Small Molecules 1
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	SM12020 Small Molecules 1

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:
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