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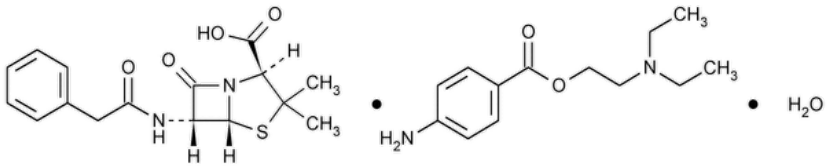
Penicillin G Procaine



$C_{16}H_{18}N_2O_4S \cdot C_{13}H_{20}N_2O_2 \cdot H_2O$ 588.72
$C_{16}H_{18}N_2O_4S \cdot C_{13}H_{20}N_2O_2$ 570.71

4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 3,3-dimethyl-7-oxo-6-[(phenylacetyl)amino-], 2*S*-(2α,5α,6β)-, compound with 2-(diethylamino)ethyl 4-aminobenzoate (1:1) monohydrate;
(2*S*,5*R*,6*R*)-3,3-Dimethyl-7-oxo-6-(2-phenylacetamido)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid compound with 2-(diethylamino)ethyl *p*-aminobenzoate (1:1) monohydrate CAS RN®: 6130-64-9; UNII: 17R794ESYN.
Anhydrous CAS RN®: 54-35-3; UNII: 1LW5K9CIR1.

DEFINITION

Penicillin G Procaine has a potency of NLT 900 Penicillin G Units/mg and NMT 1050 Penicillin G Units/mg.

IDENTIFICATION

• **A. Thin-Layer Chromatography**

Solution A: Acetone, 0.1 M citric acid, and 0.1 M sodium citrate (2:1:1)

Standard solution 1: Prepare a solution containing the equivalent of 12,000 Penicillin G Units/mL, from USP Penicillin G Potassium RS in *Solution A*.

Standard solution 2: 5 mg/mL of USP Procaine Hydrochloride RS in *Solution A*

Sample solution: Nominally 12,000 Penicillin G Units/mL from Penicillin G Procaine in *Solution A*

Chromatographic system

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

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 20 µL

Developing solvent system: Toluene, dioxane, and glacial acetic acid (90:25:4)

Spray reagent 1: Starch TS

Spray reagent 2: Iodine TS diluted 1 in 10 with water

Spray reagent 3: 50 mg/mL of *p*-dimethylaminobenzaldehyde in methanol

Analysis

Samples: *Standard solution 1*, *Standard solution 2*, and *Sample solution*

Proceed as directed in the chapter. Develop the chromatogram until the solvent has moved three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow to air-dry. Examine the plate under short- and long-wavelength UV light, noting the positions of the spots. Spray the plate with *Spray reagent 1* followed by *Spray reagent 2*. Penicillin G appears as a white spot on a purple background. Spray the location of the spots visualized with UV light with *Spray reagent 3*. Procaine appears as a bright yellow spot.

Acceptance criteria: The R_F value of the penicillin G spot from the *Sample solution* corresponds to that from *Standard solution 1*. The R_F value of the procaine spot from the *Sample solution* corresponds to that from *Standard solution 2*.

ASSAY

• **Procedure**

Standard solution: Prepare as directed in *Iodometric Assay—Antibiotics ⟨425⟩, Standard Preparation*, using USP Penicillin G Potassium RS.

Sample solution: Prepare as directed in *Iodometric Assay—Antibiotics ⟨425⟩, Assay Preparation*, except dissolve 100 mg of Penicillin G Procaine in 2.0 mL of methanol, and dilute with *Buffer B.1* (see *Antibiotics—Microbial Assays ⟨81⟩*) to obtain a solution containing 2000 Penicillin G Units/mL.

Analysis: Pipet 2 mL of the *Sample solution* into each of two glass-stoppered, 125-mL conical flasks. Use one of these to perform the *Blank Determination*. Proceed as directed in *Iodometric Assay—Antibiotics ⟨425⟩, Procedure*.

Calculate the potency, in Penicillin G Units/mg, of the Penicillin G Procaine taken:

$$\text{Result} = (B - I) \times F \times 1/(D \times V) \times 100$$

B = volume of 0.01 N sodium thiosulfate consumed in the *Blank Determination* (mL)

I = volume of 0.01 N sodium thiosulfate consumed in the *Inactivation* and *Titration* (mL)

F = equivalency factor as calculated in *Procedure* in the chapter (Penicillin G Units/mL of 0.01 N sodium thiosulfate consumed by the *Standard solution*)

D = nominal concentration of Penicillin G in the *Sample solution* (Penicillin G Units/mL)

V = volume of the *Sample solution* used for the *Inactivation* and *Titration* (mL)

Acceptance criteria: 900–1050 Penicillin G Units/mg

SPECIFIC TESTS

• **Content of Penicillin G and Procaine**

Solution A: Phosphoric acid diluted 1 in 10 with water

Mobile phase: Dissolve 14 g of monobasic potassium phosphate and 6.5 g of tetrabutylammonium hydroxide, 40% in water, in 700 mL of water. Adjust with 1 N potassium hydroxide to a pH of 7.0, and dilute with water to 1000 mL. Mix 500 mL of this solution, 250 mL of acetonitrile, and 250 mL of water. Adjust with 1 N potassium hydroxide or *Solution A* to a pH of 7.5 ± 0.05, and pass through a suitable filter.

Standard solution: 0.8 mg/mL of USP Penicillin G Potassium RS and 0.54 mg/mL of USP Procaine Hydrochloride RS in *Mobile phase*

System suitability solution: 2.4 mg/mL of USP Penicillin V Potassium RS in *Mobile phase*. Mix the resultant solution with *Standard solution* (1:3).

Sample solution: Transfer 70 mg of Penicillin G Procaine to a 50-mL volumetric flask. Add 30 mL of *Mobile phase*, sonicate to dissolve, and dilute with *Mobile phase* to volume.

Chromatographic system

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

Mode: LC

Detector: UV 235 nm

Column: 3.9-mm × 30-cm; 10-µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

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

[Note—The relative retention times for procaine, penicillin G, and penicillin V are about 0.4, 1.0, and 1.5, respectively.]

Suitability requirements

Resolution: NLT 2.0 between penicillin G and penicillin V, *System suitability solution*

Relative standard deviation: NMT 3.0% for penicillin G potassium, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of penicillin G ($C_{16}H_{18}N_2O_4S$) in the portion of Penicillin G Procaine taken:

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

r_U = peak response of penicillin G from the *Sample solution*

r_S = peak response of penicillin G from the *Standard solution*

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

C_U = concentration of Penicillin G Procaine in the *Sample solution* (mg/mL)

G_S = content of penicillin G in USP Penicillin G Potassium RS (%)

Calculate the percentage of procaine ($C_{13}H_{20}N_2O_2$) in the portion of Penicillin G Procaine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

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

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

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

C_U = concentration of Penicillin G Procaine in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of procaine, 236.32

M_{r2} = molecular weight of procaine hydrochloride, 272.78

Acceptance criteria: See *Table 1*.

Table 1

Penicillin G	51.0%–59.6%
Procaine	37.5%–43.0%

• **Bacterial Endotoxins Test ⟨85⟩:** Where the label states that Penicillin G Procaine is sterile or that it must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.01 USP Endotoxin Unit/100 Penicillin G Units.

• **Sterility Tests ⟨71⟩:** Where the label states that Penicillin G Procaine is sterile, it meets the requirements. If the test for *Membrane Filtration* is used, perform the procedure as directed in the chapter with the following exceptions. Use *Fluid A* to which has been added sufficient sterile penicillinase to inactivate the penicillin G, and swirl the vessel until solution is complete before filtering.

• **Crystallinity ⟨695⟩:** Meets the requirements

• **pH ⟨791⟩**

Sample solution: A saturated solution containing about 300 mg/mL of Penicillin G Procaine in water

Acceptance criteria: 5.0–7.5

• **Water Determination ⟨921⟩, *Method I*:** 2.8%–4.2%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Where it is intended for use in preparing injectable dosage forms, preserve as directed in *Packaging and Storage Requirements ⟨659⟩, Injection Packaging, Packaging for constitution*.

• **Labeling:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

• **USP Reference Standards ⟨11⟩**

USP Penicillin G Potassium RS
USP Penicillin V Potassium RS
USP Procaine Hydrochloride RS

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

Topic/Question	Contact	Expert Committee
PENICILLIN G PROCAINE	Ying Han Associate Science & Standards Liaison	BIO42020 Biologics Monographs 4 - Antibiotics
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	BIO42020 Biologics Monographs 4 - Antibiotics

Chromatographic Database Information: Chromatographic Database

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