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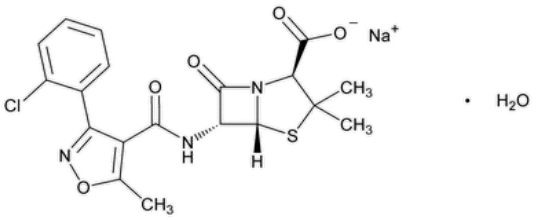
Cloxacillin Sodium



$C_{19}H_{17}ClN_3NaO_5S \cdot H_2O$ 475.88

4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 6-[[[3-(2-chlorophenyl)-5-methyl-4-isoxazolyl]carbonyl]amino]-3,3-dimethyl-7-oxo-, monosodium salt, monohydrate, [2*S*-(2α,5α,6β)]-;

Monosodium (2*S*,5*R*,6*R*)-6-[3-(*o*-chlorophenyl)-5-methyl-4-isoxazolecarboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate monohydrate CAS RN®: 7081-44-9; UNII: 65LCB00B4Y.

Anhydrous

$C_{19}H_{17}ClN_3NaO_5S$ 457.87 CAS RN®: 642-78-4; UNII: MWQ645MKMF.

DEFINITION

Cloxacillin Sodium contains the equivalent of NLT 825 µg/mg of cloxacillin ($C_{19}H_{18}ClN_3O_5S$).

IDENTIFICATION

Change to read:

• **A.** ▲Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

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

• **C.** Identification Tests—General, *Sodium* ⟨191⟩: Meets the requirements

ASSAY

• **Procedure**

Protect solutions containing cloxacillin from light.

Solution A: 1.18 g/L of sodium 1-hexanesulfonate monohydrate and 0.8 mL/L of ammonium hydroxide in water, adjusted with phosphoric acid to a pH of 2.9–3.1

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	45	55
2	45	55
2.5	35	65
5	35	65

Return to the original conditions and re-equilibrate the system.

Diluent: Acetonitrile and water (50:50)

System suitability stock solution: 0.1 mg/mL of USP Cloxacillin Related Compound D RS in *Diluent*. Sonicate as needed to dissolve.

System suitability solution: 0.001 mg/mL of USP Cloxacillin Related Compound D RS from *System suitability stock solution* and 0.1 mg/mL of USP Cloxacillin Sodium RS in *Diluent*. Store this solution at 4°.

Standard solution: 0.1 mg/mL of USP Cloxacillin Sodium RS in *Diluent*. Sonicate as needed to dissolve. Store this solution at 4°.

Sample solution: 0.1 mg/mL of Cloxacillin Sodium in *Diluent*. Sonicate as needed to dissolve. Store this solution at 4°.

Chromatographic system

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

Mode: LC

Detector: UV 225 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Temperatures

Column: 40°

Autosampler: 4°

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

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

[NOTE—The relative retention times for cloxacillin and cloxacillin related compound D are about 1.0 and 1.1, respectively.]

Suitability requirements

Resolution: NLT 1.5 between cloxacillin and cloxacillin related compound D, *System suitability solution*

Tailing factor: 0.8–1.5, *Standard solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the quantity, in µg/mg, of cloxacillin ($C_{19}H_{18}ClN_3O_5S$) in the portion of Cloxacillin Sodium taken:

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

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

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

C_U = concentration of Cloxacillin Sodium in the *Sample solution* (mg/mL)

P = potency of cloxacillin in USP Cloxacillin Sodium RS (µg/mg)

Acceptance criteria: NLT 825 µg/mg

IMPURITIES

• **ORGANIC IMPURITIES**

Protect solutions containing cloxacillin from light.

Solution A, Solution B, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	80	20
30	35	65

Return to the original conditions and re-equilibrate the system.

System suitability stock solution: 0.1 mg/mL of USP Cloxacillin Related Compound D RS in *Diluent*

System suitability solution: 0.01 mg/mL of USP Cloxacillin Related Compound D RS from *System suitability stock solution* and 1 mg/mL of USP Cloxacillin Sodium RS in *Diluent*. Store this solution at 4°.

Standard solution: 0.01 mg/mL of USP Cloxacillin Sodium RS in *Diluent*. Sonicate as needed to dissolve. Store this solution at 4°.

Sample solution: 1 mg/mL of Cloxacillin Sodium in *Diluent*. Sonicate as needed to dissolve. Store this solution at 4°.

System suitability

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

Suitability requirements

Resolution: NLT 1.5 between cloxacillin related compound D and cloxacillin, *System suitability solution*

Tailing factor: 0.8–1.5, *Standard solution*

Relative standard deviation: NMT 2.5%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Cloxacillin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (F_1/F_2) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response from the *Standard solution*

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

C_U = concentration of Cloxacillin Sodium in the *Sample solution* (mg/mL)

P = potency of cloxacillin in USP Cloxacillin Sodium RS (µg/mg)

F_1 = conversion factor, 0.001 mg/µg

F_2 = relative response factor (see *Table 3*)

Acceptance criteria: See *Table 3*. The reporting threshold is 0.05%.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Amoxicillin related compound A[a]	0.12	0.24	1.0
Cloxacillin penicilloic acid[b]	0.49	0.65	1.0
Cloxacillin penilloic acid[c,d]	0.70	1.0	1.0
	0.72		
Cloxacillin related compound D[e]	0.89	1.0	1.0
Cloxacillin	1.0	—	—
Tiocloxacillin[f]	1.18	1.0	1.0
Cloxacillin penicillamide[g]	1.25	1.0	1.0

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Cloxacillin penicilloic penicillamide[h]	1.54	1.0	1.0
Any individual unspecified impurity	—	1.0	1.0
Total impurities	—	—	5.0

[a] 6-Aminopenicillanic acid; (2*S*,5*R*,6*R*)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

[b] (4*S*)-2-{Carboxy[3-(2-chlorophenyl)-5-methylisoxazole-4-carboxamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[c] (4*S*)-2-{[3-(2-Chlorophenyl)-5-methylisoxazole-4-carboxamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[d] The system resolves two isomers. The limit is for the sum of the isomers.

[e] 3-(2-Chlorophenyl)-5-methylisoxazole-4-carboxylic acid.

[f] (2*R*,5*R*,6*R*)-6-[3-(2-Chlorophenyl)-5-methylisoxazole-4-carboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carbothioic *S*-acid.

[g] (2*S*,5*R*,6*R*)-6-{(2*S*,5*R*,6*R*)-6-[3-(2-Chlorophenyl)-5-methylisoxazole-4-carboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

[h] (2*S*,5*R*,6*R*)-6-{(*R*)-2-[(2*R*,4*S*)-4-Carboxy-5,5-dimethylthiazolidin-2-yl]-2-[3-(2-chlorophenyl)-5-methylisoxazole-4-carboxamido]acetamido}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

• **Dimethylaniline ⟨223⟩:** Meets the requirements

SPECIFIC TESTS

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

• **pH ⟨791⟩**

Sample solution: 10 mg/mL in water

Acceptance criteria: 4.5–7.5

• **Sterility Tests ⟨71⟩:** Meets the requirements where the label states that Cloxacillin Sodium is sterile. If the test for *Direct Inoculation of the Culture Medium* is used, perform the procedure as directed in the chapter with the following exceptions. Use Fluid Thioglycollate Medium containing polysorbate 80 solution (1 in 200) and an amount of sterile penicillinase sufficient to inactivate the cloxacillin in each tube. Use Soybean–Casein Digest Medium containing polysorbate 80 solution (1 in 200) and an amount of sterile penicillinase sufficient to inactivate the cloxacillin in each tube. Shake the tubes once daily.

• **Water Determination, *Method I* ⟨921⟩:** 3.0%–5.0%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers, and store at a temperature not exceeding 25°.

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

• **USP Reference Standards ⟨11⟩**

USP Cloxacillin Related Compound D RS

3-(2-Chlorophenyl)-5-methylisoxazole-4-carboxylic acid.

$C_{11}H_8ClNO_3$ 237.64

USP Cloxacillin Sodium RS

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

Topic/Question	Contact	Expert Committee
CLOXACILLIN SODIUM	Documentary Standards Support	SM12020 Small Molecules 1

Chromatographic Database Information: Chromatographic Database

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