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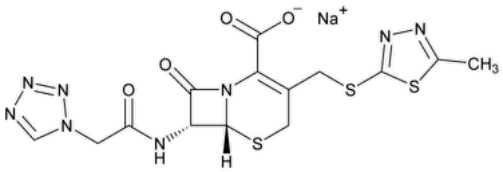
Cefazolin Sodium



$C_{14}H_{13}N_8NaO_4S_3$ 476.49

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 3-[[(5-methyl-1,3,4-thiadiazol-2-yl)thio]methyl]-8-oxo-7-[[(1*H*-tetrazol-1-yl)acetyl]amino]-, monosodium salt (6*R*-*trans*)-;

Monosodium (6*R*,7*R*)-3-[[(5-methyl-1,3,4-thiadiazol-2-yl)thio]methyl]-8-oxo-7-[2-(1*H*-tetrazol-1-yl)acetamido]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate; CAS RN®: 27164-46-1; UNII: P380M0454Z.

DEFINITION

Cefazolin Sodium has a potency equivalent to NLT 89.1% and NMT 110.1% of cefazolin sodium ($C_{14}H_{13}N_8NaO_4S_3$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

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

Solution A: Acetone and water (9:1)

Standard: USP Cefazolin RS

Sample: 150 mg

Analysis: Dissolve the *Sample* in 5 mL of water, add 0.5 mL of 2 N acetic acid, swirl, and allow to stand for 10 min in an ice bath. Filter the precipitate, and rinse with 1–2 mL of water. Dissolve in *Solution A*, evaporate the solvent almost to dryness, and dry in an oven at 60° for 30 min.

Acceptance criteria: Meets the requirements

• **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 ⟨191⟩, *Chemical Identification Tests, Sodium*:** Meets the requirements

ASSAY

• **Procedure**

Protect all solutions containing cefazolin from light.

Buffer A: 0.9 g/L of anhydrous dibasic sodium phosphate and 1.3 g/L of citric acid in water

Buffer B: 5.7 g/L of anhydrous dibasic sodium phosphate and 3.6 g/L of monobasic potassium phosphate in water

Mobile phase: Acetonitrile and *Buffer A* (1:9). Pass through a suitable filter.

Standard solution: 50 µg/mL of USP Cefazolin RS in *Buffer B*

Sample solution: 50 µg/mL of Cefazolin Sodium in *Buffer B*

Chromatographic system

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

Mode: LC

Detector: UV 254 nm

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

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of cefazolin sodium ($C_{14}H_{13}N_8NaO_4S_3$) in the portion of Cefazolin Sodium taken:

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

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

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

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

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

M_{r1} = molecular weight of cefazolin sodium, 476.49

M_{r2} = molecular weight of cefazolin, 454.51

P = potency of USP Cefazolin RS (mg/mg)

Acceptance criteria: 89.1%–110.1% on the anhydrous basis

IMPURITIES

• **Organic Impurities**

Protect all solutions containing cefazolin from light.

Solution A: 6.8 g/L of monobasic potassium phosphate

Solution B: 6.8 g/L of monobasic potassium phosphate adjusted with 10% sodium hydroxide to a pH of 6.8 before final dilution

Solution C: Acetonitrile and *Solution A* (1:1)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution B (%)	Solution C (%)
0	98	2
7	98	2
15	85	15
30	80	20
35	80	20
45	50	50
50	50	50
55	98	2
65	98	2

Blank: *Solution B*

System suitability stock solution: 2 mg/mL of USP Cefazolin RS in 0.05 M sodium hydroxide. Set the solution aside at room temperature for 5 min. [Note—The cefazolin epimer is formed upon treatment of cefazolin with sodium hydroxide.]

System suitability solution: *System suitability stock solution* and *Solution B* (1:24)

Standard solution: 25 µg/mL of USP Cefazolin RS in *Solution B*. Use this solution promptly after preparation.

Sample solution: 2.5 mg/mL of Cefazolin Sodium in *Solution B*. Use this solution promptly after preparation.

Chromatographic system

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

Mode: LC

Detector: UV 210 and 254 nm

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

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 8.0 between cefazolin and cefazolin epimer at 254 nm

Analysis

Samples: *Blank*, *Standard solution*, and *Sample solution*

Calculate the percentage of tetrazolylacetic acid and tetrazolylacetamide acetal in the portion of Cefazolin Sodium taken:

$$\text{Result} = (r_{U(210)}/r_{S(254)}) \times (C_S/C_U) \times (1/F) \times 100$$

$r_{U(210)}$ = peak response of tetrazolylacetic acid or tetrazolylacetamide acetal at 210 nm from the *Sample solution*

$r_{S(254)}$ = peak response of cefazolin at 254 nm from the *Standard solution*

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

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

F = relative response factor (see *Table 2*)

Calculate the percentage of each impurity other than tetrazolylacetic acid and tetrazolylacetamide acetal in the portion of Cefazolin Sodium taken:

$$\text{Result} = (r_{U(254)}/r_{S(254)}) \times (C_S/C_U) \times (1/F) \times 100$$

$r_{U(254)}$ = peak response of each impurity other than tetrazolylacetic acid and tetrazolylacetamide acetal at 254 nm from the *Sample solution*

$r_{S(254)}$ = peak response of cefazolin at 254 nm from the *Standard solution*

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

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

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard peaks corresponding to those in the *Blank*.

Table 2

Name	Analytical Wavelength (nm)	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Tetrazolylacetic acid[a]	210	0.07	0.40	1.0
Tetrazolylacetamide acetal[b]	210	0.08	0.33	1.0
Cefazolin open-ring lactone[c,d] or cefazolin 3-hydroxymethyl[c,e]	254	0.20	1.0	0.5
Methylthiadiazole thiol[f]	254	0.23	0.91	1.0
7-Aminocephalo sporanic acid[g]	254	0.42	1.1	1.0
Cefazolin 3-methyl analog[h]	254	0.44	0.87	1.0
Cefazolin lactone[i]	254	0.50	0.85	1.0

Name	Analytical Wavelength (nm)	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Cefazolin acetoxy analog[j]	254	0.61	0.68	1.0
Cefazolin deacylated[k]	254	0.68	1.2	1.0
Cefazoloic acid isomers[l]	254	0.84	1.0	1.0
Cefazolin	254	1.0	—	—
Cefazolin epimer[m]	254	1.2	0.98	1.0
Cefazolin pivaloyl[n]	254	1.4	0.92	1.0
Any individual unspecified impurity	254	—	1.0	0.1
Total impurities	—	—	—	3.5

[a] 2-(1*H*-Tetrazol-1-yl)acetic acid.

[b] *N*-(2,2-Dihydroxyethyl)-2-(1*H*-tetrazol-1-yl)acetamide.

[c] The identification of this impurity is tentative. The names of the most likely compounds are listed in footnotes [d] and [e].

[d] (*R*)-2-[2-(1*H*-Tetrazol-1-yl)acetamido]-2-[(*R*)-7-oxo-2,4,5,7-tetrahydro-1*H*-furo[3,4-*d*][1,3]thiazin-2-yl]acetic acid.

[e] (6*R*,7*R*)-7-[2-(1*H*-Tetrazol-1-yl)acetamido]-3-(hydroxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

[f] 5-Methyl-1,3,4-thiadiazole-2-thiol (MMTD).

[g] (6*R*,7*R*)-3-(Acetoxymethyl)-7-amino-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (7-ACA).

[h] (6*R*,7*R*)-7-[2-(1*H*-Tetrazol-1-yl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

[i] *N*-{(5a*R*,6*R*)-1,7-Dioxo-1,3,4,5a,6,7-hexahydroazeto[2,1-*b*]furo[3,4-*d*][1,3]thiazin-6-yl}-2-(1*H*-tetrazol-1-yl)acetamide.

[j] (6*R*,7*R*)-7-[2-(1*H*-Tetrazol-1-yl)acetamido]-3-(acetoxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

[k] (6*R*,7*R*)-7-Amino-3-[(5-methyl-1,3,4-thiadiazol-2-ylthio)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

[l] Three isomers of this impurity may not be fully resolved by this method. The limit applies to the sum of the isomers, which are as follows: Cefazolin open-ring delta-3: (2*R*)-2-{(*R*)-[2-(1*H*-tetrazol-1-yl)acetamido](carboxy)methyl}-5-[(5-methyl-1,3,4-thiadiazol-2-ylthio)methyl]-3,6-dihydro-2*H*-1,3-thiazine-4-carboxylic acid. Cefazolin open-ring delta-2: (2*R*)-2-{(*R*)-[2-(1*H*-tetrazol-1-yl)acetamido](carboxy)methyl}-5-[(5-methyl-1,3,4-thiadiazol-2-ylthio)methyl]-3,4-dihydro-2*H*-1,3-thiazine-4-carboxylic acid. Cefazolin open-ring delta-4: (2*R*)-2-{(*R*)-[2-(1*H*-tetrazol-1-yl)acetamido](carboxy)methyl}-5-[(5-methyl-1,3,4-thiadiazol-2-ylthio)methyl]-5,6-dihydro-2*H*-1,3-thiazine-4-carboxylic acid.

[m] (6*R*,7*S*)-7-[2-(1*H*-Tetrazol-1-yl)acetamido]-3-[(5-methyl-1,3,4-thiadiazol-2-ylthio)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

[n] (6*R*,7*R*)-3-((5-Methyl-1,3,4-thiadiazol-2-ylthio)methyl)-8-oxo-7-pivalamido-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

SPECIFIC TESTS

• **Optical Rotation** ⟨781S⟩, *Procedures, Specific Rotation*
Sample solution: 55 mg/mL, in 0.1 M sodium bicarbonate
Acceptance criteria: −10° to −24°

• **pH** ⟨791⟩
Sample solution: 100 mg/mL of cefazolin
Acceptance criteria: 4.0–6.0

• **Water Determination** ⟨921⟩, *Method I*: NMT 6.0%

• **Sterility Tests** ⟨71⟩, *Test for Sterility of the Product to Be Examined, Membrane Filtration*: Where the label states that Cefazolin Sodium is sterile, it meets the requirements.

• **Bacterial Endotoxins Test** ⟨85⟩: Where the label states that Cefazolin Sodium is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.15 USP Endotoxin Units/mg of cefazolin.

ADDITIONAL REQUIREMENTS

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

• **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 Cefazolin RS

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

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

Chromatographic Database Information: Chromatographic Database

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