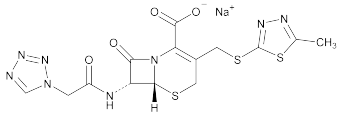
Cefazolin Sodium



$C_{14}H_{13}N_8NaO_4S_3$ 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 (*6R-trans*);
Monosodium (*6R,7R*)-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 [27164-46-1].

DEFINITION

Cefazolin Sodium has a potency equivalent to NLT 89.1% and NMT 110.1% of cefazolin sodium ($C_{14}H_{13}NaN_8O_4S_3$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. Ultraviolet Absorption** ⟨**197U**⟩
  Sample solution: 20 µg/mL in 0.1 M sodium bicarbonate
- **B.** The retention time of the major peak for cefazolin in 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**
  Buffer A: 0.9 g/L of anhydrous dibasic sodium phosphate and 1.298 g/L of citric acid monohydrate in water
  Buffer B: 5.68 g/L of anhydrous dibasic sodium phosphate and 3.63 g/L of monobasic potassium phosphate in water
  Mobile phase: Acetonitrile and *Buffer A* (1:9). Pass through a membrane filter having a 10-µm or finer pore size.
  Internal standard solution: 7.5 mg/mL of salicylic acid in methanol and *Buffer B* (1:9). Dissolve first in methanol, using 10% of the final volume, and dilute with water to volume.
  Standard stock solution: 1 mg/mL of USP Cefazolin RS in *Buffer B*
  Standard solution: 50 µg/mL of cefazolin from the *Standard stock solution* and 0.4 mg/mL of salicylic acid from the *Internal standard solution* in *Buffer B*
  Sample stock solution: 1 mg/mL of Cefazolin Sodium in *Buffer B*
  Sample solution: 50 µg/mL of cefazolin sodium from the *Sample stock solution* and 0.4 mg/mL of salicylic acid from the *Internal standard solution* in *Buffer B*
  Chromatographic system
  (See *Chromatography* ⟨621⟩*, System Suitability*.)
  Mode: LC
  Detector: UV 254 nm
  Column: 4.0-mm × 30-cm; 10-µm packing L1
  Flow rate: 2 mL/min
  Injection size: 10 µL
  System suitability
  Sample: *Standard solution*
  [NOTE—The relative retention times for salicylic acid and cefazolin are about 0.7 and 1.0, respectively.]
  Suitability requirements
  Resolution: NLT 4.0 between the analyte and the internal standard peaks
  Column efficiency: NLT 1500 theoretical plates
  Tailing factor: NMT 1.5
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of cefazolin sodium ($C_{14}H_{13}NaN_8O_4S_3$) in the portion of Cefazolin Sodium taken:

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

R_U = peak response ratio of cefazolin to the internal standard from the *Sample solution*
R_S = peak response ratio of cefazolin to the internal standard from the *Standard solution*
C_S = concentration of USP Cefazolin RS, calculated on the anhydrous basis, in the *Standard solution* (mg/mL)
C_U = nominal 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

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

IMPURITIES

- **Organic Impurities**
  [NOTE—Use the *Sample solution* immediately after preparation.]
  Buffer A: 6.8 g/L of monobasic potassium phosphate
  Buffer B: 6.8 g/L of monobasic potassium phosphate adjusted with 10% sodium hydroxide to a pH of 6.8 prior to final dilution
  Solution C: Acetonitrile and *Buffer A* (1:1)
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Buffer 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: Use *Buffer 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 *Buffer B* (1:24)
Standard solution: 25 µg/mL of USP Cefazolin RS in *Buffer B*
Sample solution: 2.5 mg/mL of Cefazolin Sodium in *Buffer B*
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
Temperature: 30°
Flow rate: 1.5 mL/min
Injection size: 20 µL

System suitability
Sample: *System suitability solution*
Suitability requirements
Resolution: NLT 8.0 between cefazolin and cefazolin epimer, 254 nm

Analysis
Samples: *Blank*, *Standard solution*, and *Sample solution*. Disregard peaks corresponding to those in the *Blank*.
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:

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

r_U = peak response of each impurity other than tetrazolylacetic acid and tetrazolylacetamide acetal at 254 nm from the *Sample solution*
r_S = 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
Individual impurities: See *Table 2*.

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
[c]Cefazolin open-ring lactone[d] or Cefazolin 3-hydroxymethyl[e]	254	0.20	1.0	0.5
Methylthiadiazole thiol[f]	254	0.23	0.91	1.0
7-Aminocephalosporanic 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
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]● (RB 1-Jan-2011)	254	0.84	1.0	●1.0● (RB 1-Jan-2011)
Cefazolin	254	1.0	—	—
Cefazolin epimer[m]	254	1.2	0.98	1.0
Cefazolin pivaloyl[n]	254	1.4	0.92	1.0

[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.● (RB 1-Jan-2011)
[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.

Table 2 *(Continued)*

Name	Analytical Wavelength (nm)	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
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. ● (RB 1-Jan-2011)
[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,** *Specific Rotation* ⟨**781S**⟩**:** –10° to –24°
  Sample solution: 55 g/L, in 0.1 M sodium bicarbonate
- **pH** ⟨**791**⟩**:** 4.0–6.0, in a solution containing 100 mg/mL of cefazolin
- **Water Determination,** *Method I* ⟨**921**⟩**:** NMT 6.0%
- **Sterility Tests** ⟨**71**⟩**:** Where the label states that Cefazolin Sodium is sterile, it meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
- **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 Unit/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
  USP Endotoxin RS