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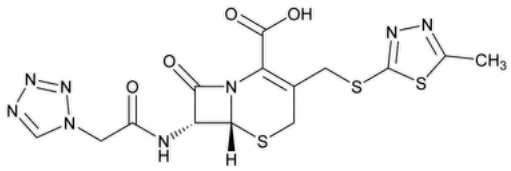
Cefazolin



$C_{14}H_{14}N_8O_4S_3$ 454.51

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]-(6*R*-*trans*);
(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-carboxylic acid CAS RN®: 25953-19-9; UNII: IHS69L0Y4T.

DEFINITION

Cefazolin contains NLT 95.0% and NMT 103.0% of cefazolin ($C_{14}H_{14}N_8O_4S_3$), calculated on the anhydrous basis.

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*.

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 monohydrate 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* (10:90)

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

Sample solution: 50 µg/mL of Cefazolin 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

Relative standard deviation: NMT 2.0%

Tailing factor: NMT 1.5

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of cefazolin ($C_{14}H_{14}N_8O_4S_3$) in the portion of Cefazolin taken:

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

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 in the *Sample solution* (mg/mL)

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

Acceptance criteria: 95.0%–103.0% on the anhydrous basis

IMPURITIES

• **Organic Impurities**

Protect all solutions containing cefazolin from light.

Solution A: 6.8 g/L of monobasic potassium phosphate in water

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

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: Use *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 immediately after preparation.

Sample solution: 2.5 mg/mL of Cefazolin in *Solution B*. Use this solution immediately 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, 254 nm

Analysis

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

Calculate the percentage of tetrazolylacetic acid and tetrazolylacetamide acetal in the portion of Cefazolin 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 the Cefazolin 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 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 or 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 the Cefazolin 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
Tetrazolyl acetamide acetal[b]	210	0.08	0.33	1.0
Cefazolin open-ring lactone[c,d] or Cefazolin 3-hydroxymethyl[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
Cefazolin acetoxy analog[j]	254	0.61	0.68	1.0
Cefazolin deacyl ated[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

Name	Analytical Wavelength (nm)	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
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

• **Water Determination,** *Method I* ⟨921⟩**:** NMT 2.0%

ADDITIONAL REQUIREMENTS

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

• **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	Documentary Standards Support	SM12020 Small Molecules 1

Chromatographic Database Information: Chromatographic Database

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