Assay preparation 2 (where the label states the quantity of cefamandole in a given volume of constituted solution)—Constitute Cefamandole Nafate for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Quantitatively dilute an accurately measured volume of the constituted solution with water to obtain a solution containing about 2 mg of cefamandole per mL. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, add 30.0 mL of *pH 2.3 Buffer,* dilute with water to volume, and mix.

Assay preparation 3—Using an accurately weighed quantity of Cefamandole Nafate for Injection, prepare as directed for *Standard preparation* under *Cefamandole Nafate.* Determine the sodium carbonate content of a separate, accurately weighed, 1-g portion of Cefamandole Nafate for Injection dissolved in 100 mL of water. Add methyl orange TS, and titrate with 0.2 N sulfuric acid VS. Each mL of 0.2 N sulfuric acid is equivalent to 10.60 mg of Na_2CO_3.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Cefamandole Nafate.* Calculate the quantity, in mg, of cefamandole ($C_{18}H_{18}N_6O_5S_2$) in the portion of constituted solution taken by the formula:

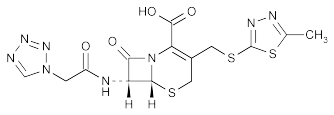
$$(CP)(L / 1000D)(i_U / i_S)$$

in which C is the concentration, in mg per mL, of USP Cefamandole Nafate RS in the *Standard preparation; L* is the labeled quantity, in mg, in the portion of constituted solution taken; D is the concentration, in mg per mL, of cefamandole in *Assay preparation 1* or in *Assay preparation 2,* based on the volume of constituted solution taken and the extent of dilution; and the other terms are as defined therein. Calculate the potency, in µg of cefamandole ($C_{18}H_{18}N_6O_5S_2$) per mg, of the Cefamandole Nafate for Injection taken by the formula:

$$(CP / W)(i_U / i_S)$$

in which W is the weight, in mg, of the Cefamandole Nafate for Injection taken in each mL of *Assay preparation 3,* and the other terms are as defined therein. Where the test for *Uniformity of dosage units* has been performed using the *Procedure for content uniformity,* use the average of these determinations as the *Assay* value.

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,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-carboxylic acid [25953-19-9].

» Cefazolin contains not less than 95.0 per cent and not more than 103.0 per cent of $C_{14}H_{14}N_8O_4S_3$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩**—**
USP Cefazolin RS

Identification—The retention time of the major peak for cefazolin in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay*.

Water, *Method I* ⟨921⟩: not more than 2.0%.

Heavy metals, *Method II* ⟨231⟩: 0.002%.

Assay—

pH 3.6 Buffer—Dissolve 0.900 g of anhydrous dibasic sodium phosphate and 1.298 g of citric acid monohydrate in water to make 1000 mL.

pH 7.0 Buffer—Dissolve 5.68 g of anhydrous dibasic sodium phosphate and 3.63 g of monobasic potassium phosphate in water to make 1000 mL.

Mobile phase—Prepare a suitable mixture of *pH 3.6 Buffer* and acetonitrile (9:1). Pass through a membrane filter having a 10-µm or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution—Transfer 750 mg of salicylic acid to a 100-mL volumetric flask, dissolve in 10 mL of methanol, dilute with *pH 7.0 Buffer* to volume, and mix.

Standard preparation—Transfer about 25 mg of USP Cefazolin RS, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with *pH 7.0 Buffer* to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with *pH 7.0 Buffer* to volume, and mix.

Assay preparation—Proceed as directed for *Standard preparation*, except to use about 25 mg of Cefazolin, accurately weighed.

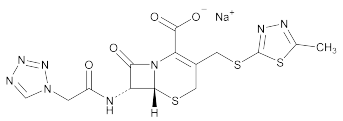
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.0-mm × 30-cm column that contains 10- µm packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.7 for salicylic acid and 1.0 for cefazolin; the resolution, R, between the analyte and internal standard peaks is not less than 4.0; the column efficiency is not less than 1500 theoretical plates; the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{14}H_{14}N_8O_4S_3$ in the portion of Cefazolin taken by the formula:

$$500C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Cefazolin RS, calculated on the anhydrous basis, in the *Standard preparation*; and R_U and R_S are the peak response ratios of cefazolin to the internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively.

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,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 NL T 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
  Acceptance criteria: Meets the requirements
- **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 ($C_{14}H_{13}N_8NaO_4S_3$) in the portion of Cefazolin Sodium taken:

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

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
  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 *Buffer 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 *Buffer B* (1:24)
Standard solution: 25 µg/mL of USP Cefazolin RS in *Solution B*
Sample solution: 2.5 mg/mL of Cefazolin Sodium in *Solution 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*
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*)

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]	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.

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*.

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨**781S**⟩
Sample solution: 55 mg/mL, in 0.1 M sodium bicarbonate
Acceptance criteria: –10° to –24°
- **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

Cefazolin Injection

» Cefazolin Injection is a sterile solution of Cefazolin and Sodium Bicarbonate in a diluent containing one or more suitable tonicity-adjusting agents. It contains not less than 90.0 per cent