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

and not more than 115.0 per cent of the labeled amount of cefazolin ($C_{14}H_{14}N_8O_4S_3$).

Packaging and storage—Preserve in *Containers for Injections* as described under *Injections* ⟨1⟩. Maintain in the frozen state.

Labeling—It meets the requirements for *Labeling* under *Injections* ⟨1⟩. The label states that it is to be thawed just prior to use, describes conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.

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

Bacterial endotoxins ⟨85⟩—It contains not more than 0.15 USP Endotoxin Unit per mg of cefazolin.

Sterility ⟨71⟩—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

pH ⟨791⟩: between 4.5 and 7.0.

Particulate matter ⟨788⟩: meets the requirements for small-volume injections.

Assay—

pH 3.6 Buffer, pH 7.0 Buffer, Mobile phase, Internal standard solution, Standard preparation, and *Chromatographic system*—Prepare as directed in the *Assay* under *Cefazolin*.

Assay preparation—Allow 1 container of Injection to thaw, and mix. Transfer an accurately measured volume of the Injection, equivalent to about 50 mg of cefazolin, to a 50-mL volumetric flask, 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.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Cefazolin*. Calculate the quantity, in mg, of cefazolin ($C_{14}H_{14}N_8O_4S_3$) in each mL of the Injection taken by the formula:

$$(1000C / V)(R_U / R_S)$$

in which V is the volume, in mL, of Injection taken, and the other terms are as defined therein.

Cefazolin for Injection

» Cefazolin for Injection contains an amount of Cefazolin Sodium equivalent to not less than 90.0 percent and not more than 115.0 per cent of the labeled amount of cefazolin ($C_{14}H_{14}N_8O_4S_3$).

Packaging and storage—Preserve in *Containers for Injections* as described under *Injections* ⟨1⟩.

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

Constituted solution—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* ⟨1⟩.

Identification—

A: *Ultraviolet Absorption* ⟨197U⟩—

Solution: 20 µg per mL.

Medium: 0.1 M sodium bicarbonate.

B: 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*.

C: It meets the requirements of the tests for *Sodium* ⟨191⟩.

Specific rotation ⟨781S⟩: between −10° and −24°.

Test solution: 55 mg per mL, in 0.1 M sodium bicarbonate.

Bacterial endotoxins ⟨85⟩—It contains not more than 0.15 USP Endotoxin Unit per mg of cefazolin.

Sterility ⟨71⟩—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

Uniformity of dosage units ⟨905⟩: meets the requirements.

Procedure for content uniformity—Perform the *Assay* on individual containers using *Assay preparation 1* or *Assay preparation 2*, or both, as appropriate.

pH ⟨791⟩: between 4.0 and 6.0, in a solution containing 100 mg of cefazolin per mL.

Water, *Method I* ⟨921⟩: not more than 6.0%.

Particulate matter ⟨788⟩: meets the requirements for small-volume injections.

Other requirements—It meets the requirements for *Labeling* under *Injections* ⟨1⟩.

Assay—

pH 3.6 Buffer, pH 7.0 Buffer, Mobile phase, Internal standard solution, Standard preparation, and *Chromatographic system*—Prepare as directed in the *Assay* under *Cefazolin.*

Assay preparation 1 (where it is packaged for dispensing and is represented as being in a single-dose container)—Constitute Cefazolin for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively with *pH 7.0 buffer* to obtain a stock solution containing about 1 mg of cefazolin per mL. T ransfer 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 2 (where the label states the quantity of cefazolin in a given volume of constituted solution)—Constitute Cefazolin for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively with *pH 7.0 buffer* to obtain a stock solution containing about 1 mg of cefazolin per mL. T ransfer 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.

Procedure—Proceed as directed in the *Assay* under *Cefazolin.* Calculate the quantity, in mg, of cefazolin ($C_{14}H_{14}N_8O_4S_3$) in the container, and in the volume of constituted solution taken by the formula:

$$(CL / D)(R_U / R_S)$$

in which L is the labeled quantity, in mg, of cefazolin in the container, or in the volume of constituted solution taken; D is the concentration, in mg per mL, of cefazolin in the stock solution used in preparing *Assay preparation 1* or *Assay preparation 2*, on the basis of the labeled quantity in the container, or in the volume of constituted solution taken, respectively, and the extent of dilution; 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.