Cefazolin Sodium

C₁₄H₁₃Na₂O₈S₃ 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-[[1H-tetra-
zol-1-yl]acetamido]-, monosodium salt (6R-trans);
Monosodium (6R,7R)-3-[[5-methyl-1,3,4-thiadiazol-2-yl]thio-
o]methyl]-8-oxo-7-[2-(1H-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 (C₁₄H₁₃Na₂O₈S₃), calcu-
lated 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

Table 1

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Buffer B (%)</th>
<th>Solution C (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>98</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>98</td>
<td>2</td>
</tr>
<tr>
<td>15</td>
<td>85</td>
<td>13</td>
</tr>
<tr>
<td>30</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>35</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>45</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>55</td>
<td>98</td>
<td>2</td>
</tr>
<tr>
<td>65</td>
<td>98</td>
<td>2</td>
</tr>
</tbody>
</table>

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.)

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 (C₁₄H₁₃Na-
₂O₈S₃) in the portion of Cefazolin Sodium taken:

\[
\text{Result} = \left( \frac{R_a}{R_i} \right) \times \left( \frac{C_i}{C_0} \right) \times \left( \frac{M_2}{M_1} \right) \times 100
\]

\[
R_a = \text{peak response ratio of cefazolin to the internal standard from the Sample solution}
\]
\[
R_i = \text{peak response ratio of cefazolin to the internal standard from the Standard solution}
\]
\[
C_i = \text{concentration of USP Cefazolin RS, calculated on the anhydrous basis, in the Standard solution (mg/mL)}
\]
\[
C_0 = \text{nominal concentration of Cefazolin Sodium in the Sample solution (mg/mL)}
\]
\[
M_1 = \text{molecular weight of cefazolin sodium, 476.49}
\]
\[
M_2 = \text{molecular weight of cefazolin, 454.51}
\]

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 ad-
  justed 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.

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Cefazolin

**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**

**System suitability**

<table>
<thead>
<tr>
<th>Name</th>
<th>Analytical Wavelength (nm)</th>
<th>Relative Retention Time</th>
<th>Relative Response Factor</th>
<th>Acceptance Criteria, NMT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetrazolylacetic acid</td>
<td>210</td>
<td>0.07</td>
<td>0.40</td>
<td>1.0</td>
</tr>
<tr>
<td>Tetrazolylacetamide acetal</td>
<td>210</td>
<td>0.08</td>
<td>0.33</td>
<td>1.0</td>
</tr>
<tr>
<td>Cefazolin open-ring lactone</td>
<td>254</td>
<td>0.20</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Cefazolin 3-methyl analog</td>
<td>254</td>
<td>0.23</td>
<td>0.91</td>
<td>1.0</td>
</tr>
<tr>
<td>Cefazolin lactone</td>
<td>254</td>
<td>0.44</td>
<td>0.87</td>
<td>1.0</td>
</tr>
<tr>
<td>Cefazolin acetoxyl analogue</td>
<td>254</td>
<td>0.61</td>
<td>0.68</td>
<td>1.0</td>
</tr>
<tr>
<td>Cefazolin decacylated</td>
<td>254</td>
<td>0.68</td>
<td>1.2</td>
<td>1.0</td>
</tr>
<tr>
<td>Cefazolin epimer</td>
<td>254</td>
<td>1.2</td>
<td>0.98</td>
<td>1.0</td>
</tr>
<tr>
<td>Cefazolin pivaloyl</td>
<td>254</td>
<td>1.4</td>
<td>0.92</td>
<td>1.0</td>
</tr>
</tbody>
</table>

| C_1                                   | concentration of USP Cefazolin RS in the Standard solution (mg/mL) |
| C_2                                   | concentration of Cefazolin Sodium in the Standard solution (mg/mL) |
| F                                     | relative response factor (see Table 2) |

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

\[ \text{Result} = \left( \frac{r_u}{r_S} \right) \times \left( \frac{C_u}{C_1} \right) \times \left( \frac{1}{F} \right) \times 100 \]

- **r_u** = peak response of each impurity other than tetrazolylactic 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_u** = concentration of USP Cefazolin RS in the Standard solution (mg/mL)
- **C_1** = concentration of Cefazolin Sodium in the Sample solution (mg/mL)
- **F** = relative response factor (see Table 2)

**Acceptance criteria**

| Individual impurities | See Table 2. |

**Footnotes:**
- N-(2,2-Dihydroxyethyl)-2-(1H-tetrazol-1-yl)acetamide.
- N-[5,5,6,6 octahydro azeto[2,1-b][1,3]thiazin-6-yl]-2-(1H-tetrazol-1-yl)acetamide.
- 3-(Acetoxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (7-ACA).
- 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.
- 3-((5-methyl-1,3,4-thiadiazol-2-ylthio)methyl)-6,6-dihydro-2H-1,3-thiazine-4-carboxylic acid.
- 3-((5-Methyl-1,3,4-thiadiazol-2-ythio)methyl)-8-oxo-7-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
<table>
<thead>
<tr>
<th>Name</th>
<th>Analytical Wavelength (nm)</th>
<th>Relative Retention Time</th>
<th>Relative Response Factor</th>
<th>Acceptance Criteria, NMT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any individual unspecified impurity</td>
<td>254</td>
<td>—</td>
<td>1.0</td>
<td>0.1</td>
</tr>
<tr>
<td>Total impurities</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>3.5</td>
</tr>
</tbody>
</table>

* 2-(1H-Tetrazol-1-yl)acetic acid.
* N-(2,2-Dihydroxyethyl)-2-(1H-tetrazol-1-yl)acetamide.
* The identification of this impurity is tentative. The names of the most likely compounds are listed in footnotes a and e.
* (R)-2-[2-(1H-Tetrazol-1-yl)acetamido]-2-[[R]-7-oxo-2,4,5,7-tetrahydro-1H-furo[3,4-d][1,3]thiazin-2-yl]acetic acid.
* (6R,7R)-7-[2-(1H-Tetrazol-1-yl)acetamido]-3-(hydroxymethyl)8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
* N-Methyl-1,3,4-thiadiazole-2-thiol (MMTD).
* (6R,7R)-7-(2-(1H-Tetrazol-1-yl)acetamido)-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
* (6R,7R)-2-(1H-Tetrazol-1-yl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
* 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: (2R)-2-{((R)-[2-(1H-Tetrazol-1-yl)acetamido][carboxy)methyl]-3,6-dihydro-2H-1,3-thiazine-4-carboxylic acid.
  - Cefazolin open-ring delta-2: (2R)-2-{((R)-[2-(1H-Tetrazol-1-yl)acetamido][carboxy)methyl]-3,4-dihydro-2H-1,3-thiazine-4-carboxylic acid.
  - Cefazolin open-ring delta-4: (2R)-2-{((R)-[2-(1H-Tetrazol-1-yl)acetamido][carboxy)methyl]-5,6-dihydro-2H-1,3-thiazine-4-carboxylic acid.

**SPECIFIC TESTS**
- **Optical Rotation, Specific Rotation (781S):** −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

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