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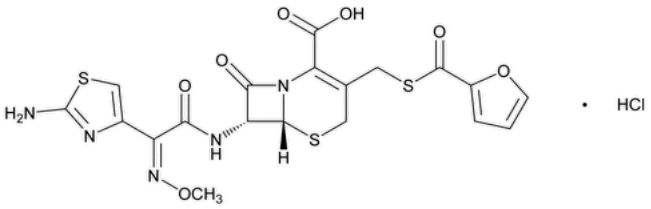
# Ceftiofur Hydrochloride



$C_{19}H_{17}N_5O_7S_3 \cdot HCl$ 560.02

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[(2-amino-4-thiazolyl)(methoxyimino)acetyl]amino]-3-[[(2-furanylcarbonyl)thio]methyl]-8-oxo-, monohydrochloride, [6*R*-[6α,7β(*Z*)]]-;

(6*R*,7*R*)-7-[2-(2-Amino-4-thiazolyl)glyoxylamido]-3-(mercaptomethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, $7^2$-(*Z*)-(*O*-methyloxime), 2-furoate (ester), monohydrochloride CAS RN®: 103980-44-5.

## DEFINITION

Ceftiofur Hydrochloride contains NLT 844 µg/mg and NMT 956 µg/mg of ceftiofur ($C_{19}H_{17}N_5O_7S_3$), calculated on the anhydrous and solvent-free basis.

## IDENTIFICATION

- **A. Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*:** 197M
- **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

***Change to read:***

- **Procedure**

**Solution A:** Tetrabutylammonium hydroxide, 40% in water

**Solution B:** Dissolve 3.85 g of ammonium acetate and 13.5 mL of *Solution A* in 700 mL of water. Adjust with glacial acetic acid to a pH of 6.7.

**Mobile phase:** Mix 700 mL of *Solution B* with 200 mL of methanol and 110 mL of tetrahydrofuran.

**Diluent:** 0.05 M ammonium acetate

**Standard solution:** 0.16 mg/mL of USP Ceftiofur Hydrochloride RS prepared as follows. Dissolve USP Ceftiofur Hydrochloride RS in methanol using about 4% of the final volume and dilute with *Diluent* to volume.

**Sample solution:** 0.16 mg/mL of Ceftiofur Hydrochloride prepared as follows. Dissolve Ceftiofur Hydrochloride in methanol using about 4% of the final volume and dilute with *Diluent* to volume.

**Chromatographic system**

(See *Chromatography ⟨621⟩, System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L7

**Column temperature:** 40°

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the ▲quantity, in µg/mg,▲ (ERR 1-Oct-2022) of ceftiofur ($C_{19}H_{17}N_5O_7S_3$) in the portion of Ceftiofur Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P^{\blacktriangle}{}_{\blacktriangle\ (\text{ERR 1-Oct-2022})}$$

$r_U$ = peak response from the *Sample solution*

$r_S$ = peak response from the *Standard solution*

$C_S$ = concentration of USP Ceftiofur Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$ = concentration of Ceftiofur Hydrochloride in the *Sample solution* (mg/mL)

$P$ = potency of ceftiofur in USP Ceftiofur Hydrochloride RS (µg/mg)

**Acceptance criteria:** 844–956 µg/mg on the anhydrous and solvent-free basis

## IMPURITIES

• **Low Molecular Weight Impurities**

**Solution A:** Acetonitrile, trifluoroacetic acid, and water (50:1:950)

**Solution B:** Acetonitrile, trifluoroacetic acid, and water (800:1:200)

**Mobile phase:** See *Table 1*.

**Table 1**

| Time (min) | Solution A (%) | Solution B (%) |
|---|---|---|
| 0 | 100 | 0 |
| 5 | 100 | 0 |
| 35 | 60 | 40 |
| 50 | 0 | 100 |
| 55 | 0 | 100 |
| 60 | 100 | 0 |
| 75 | 100 | 0 |

**Diluent:** Acetonitrile and water (1:1)

**System suitability solution:** 0.1 mg/mL of USP Ceftiofur System Suitability Mixture RS in *Diluent*. Sonicate as needed to dissolve. Inject within 20 min of preparation.

**Peak identification solution:** 15 µg/mL of USP Cefotaxime Sodium RS in *Diluent*

**Sample stock solution:** 3 mg/mL of Ceftiofur Hydrochloride in *Diluent*

**Sample solution:** 0.3 mg/mL of Ceftiofur Hydrochloride from *Sample stock solution* in water. Inject the *Sample solution* within 20 min of preparation.

**Chromatographic system**

(See *Chromatography ⟨621⟩, System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 15-cm; 3-µm packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 10 µL

**System suitability**

**Sample:** *System suitability solution*

[Note—The relative retention times for ceftiofur delta-3 isomer and ceftiofur are 0.98 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 1.5 between ceftiofur delta-3 isomer and ceftiofur

**Analysis**

**Samples:** *Sample solution* and *Peak identification solution*

[Note—The elution order of the *N*-deacyl ceftiofur and cefotaxime peaks may be reversed depending on the column used. Determine the location of the cefotaxime peak by using the *Peak identification solution*.]

Calculate the percentage of each impurity in the portion of Ceftiofur Hydrochloride taken:

$$\text{Result} = \{r_U/[r_S + \Sigma(r_U/F)]\} \times (1/F) \times 100$$

$r_U$ = peak response of each impurity from the *Sample solution*

$r_S$ = peak response of ceftiofur from the *Sample solution*

$F$ = relative response factor (see *Table 2*)

**Acceptance criteria:** See *Table 2*. The reporting threshold is 0.05% of the total corrected peak area.

**Table 2**

| Name | Relative Retention Time | Relative Response Factor | Acceptance Criteria, NMT (%) |
|---|---|---|---|
| Aminothiazolyl oxime ethyl ester[a] | 0.03 | 1.6 | 0.5 |
| 7-Aminocephalosporanic acid[b] | 0.10 | 0.77 | 0.5 |
| 2-Furoic acid[c] | 0.33 | 2.5 | 0.5 |
| *N*-Deacyl ceftiofur[d] | 0.70 | 0.61 | 0.5 |
| Cefotaxime[e] | 0.71 | 1.0 | 0.5 |
| Ceftiofur delta-3 isomer[f] | 0.98 | 1.0 | 0.5 |
| Ceftiofur | 1.0 | — | — |
| Ceftiofur *E*-isomer[g] | 1.08 | 1.0 | 3.6 |
| Dihydrothiophenyl thioester[h] | 1.2 | 1.0 | 0.5 |
| Ceftiofur amide dimer[i] | 1.3 | 1.0 | 0.8 |
| *N*-Trityl ceftiofur oxime[j] | 1.5 | 0.48 | 0.5 |
| Any other individual impurity | — | 1.0 | 0.5 |
| Total impurities | — | — | 6.0 |

[a] (*Z*)-Ethyl 2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetate.

[b] (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.

[c] Furan-2-carboxylic acid.

[d] (6*R*,7*R*)-7-Amino-3-((furan-2-carbonylthio)methyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

[e] (6*R*,7*R*)-3-(Acetoxymethyl)-7-((*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

[f] (6*R*,7*R*)-7-((*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido)-3-((furan-2-carbonylthio)methyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylic acid.

[g] (6*R*,7*R*)-7-((*E*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido)-3-((furan-2-carbonylthio)methyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

[h] *S*-(4-Hydroxy-5-oxo-2,5-dihydrothiophen-3-yl)methyl furan-2-carbothioate.

[i] (6*R*,7*S*)-7-{(6*R*,7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-[(furan-2-carbonylthio)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-enecarboxamido}-3-[(furan-2-carbonylthio)methyl]-8-oxo-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

[j] (*Z*)-2-(Methoxyimino)-2-(2-(tritylamino)thiazol-4-yl)acetic acid.

• **High Molecular Weight Impurities (Ceftiofur Polymers)**

To minimize leaching of plastic components, avoid contact between the *Mobile phase* and plastics during all steps including during the preparation of the *Mobile phase*, the *System suitability solution*, and the *Sample solution*, and when filling the injection vial.

**Solution A:** 45% of potassium hydroxide in water

**Solution B:** 0.68 g/L of monobasic potassium phosphate. Adjust with *Solution A* to a pH of 7.5.

**Mobile phase:** 10 g/L of electrophoresis grade sodium dodecyl sulfate in *Solution B*. Stir or slightly heat to dissolve.

**Blank:** Use the *Mobile phase*.

**System suitability solution:** 0.15 mg/mL of USP Ceftiofur System Suitability Mixture RS in *Mobile phase*. Sonicate if necessary to dissolve. Inject within 20 min of preparation.

**Sample solution:** 0.15 mg/mL of Ceftiofur Hydrochloride in *Mobile phase*. Inject within 20 min of preparation.

**Chromatographic system**

(See *Chromatography ⟨621⟩, System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.0-mm × 25-cm; 5-µm packing L20

**Flow rate:** 1 mL/min

**Injection volume:** 20 µL

**Run time:** NLT 2 times the retention time of the ceftiofur peak

**System suitability**

**Sample:** *System suitability solution*

[Note—Adjust the chromatographic system so that the retention time of ceftiofur is NLT 2.5 min. The relative retention times of ceftiofur *E*-isomer and ceftiofur are 0.9 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 1.0 between ceftiofur and ceftiofur *E*-isomer

**Analysis**

**Samples:** *Blank* and *Sample solution*

Calculate the percentage of high molecular weight impurities in the portion of Ceftiofur Hydrochloride taken:

$$\text{Result} = \{100 \times (r_U/F)/[(r_U/F) + r_C + r_A]\} - T$$

$r_U$ = sum of the responses of all peaks that elute prior to ceftiofur from the *Sample solution*, corrected for the *Blank* if necessary

$F$ = relative response factor, 0.8

$r_C$ = peak response of ceftiofur from the *Sample solution*

$r_A$ = sum of the responses of all peaks that elute after ceftiofur from the *Sample solution*

$T$ = total impurities from *Table 2*

**Acceptance criteria**

**Total high molecular weight impurities:** NMT 3.9%

## SPECIFIC TESTS

• **Optical Rotation ⟨781S⟩, *Procedures, Specific Rotation***

**Sample solution:** 5 mg/mL in dimethylformamide

**Acceptance criteria:** −115° to −127° on the anhydrous and solvent-free basis

• **Bacterial Endotoxins Test ⟨85⟩:** Where the label states that Ceftiofur Hydrochloride is sterile or that it must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 1.0 USP Endotoxin Unit/mg of ceftiofur hydrochloride.

• **Water Determination ⟨921⟩, *Method I*:** NMT 6.0%

## ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight, light-resistant containers. Store in a freezer.

• **Labeling:** Label it to indicate that it is intended for veterinary use only. 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 Cefotaxime Sodium RS

USP Ceftiofur Hydrochloride RS

USP Ceftiofur System Suitability Mixture RS

This is a mixture of ceftiofur, ceftiofur delta-3 isomer, ceftiofur *E*-isomer, and other impurities.

Ceftiofur delta-3 isomer

(6*R*,7*R*)-7-((*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido)-3-((furan-2-carbonylthio)methyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylic acid.

$C_{19}H_{17}N_5O_7S_3$ 523.56

Ceftiofur *E*-isomer

(6*R*,7*R*)-7-((*E*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido)-3-((furan-2-carbonylthio)methyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

$C_{19}H_{17}N_5O_7S_3$ 523.56

USP Endotoxin RS

**Auxiliary Information** - Please check for your question in the FAQs before contacting USP.

| Topic/Question | Contact | Expert Committee |
|---|---|---|
| CEFTIOFUR HYDROCHLORIDE | Documentary Standards Support | SM32020 Small Molecules 3 |

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