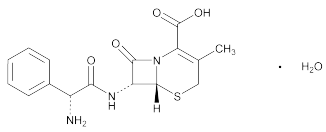
Cephalexin



$C_{16}H_{17}N_3O_4S \cdot H_2O$ 365.40

$C_{16}H_{17}N_3O_4S$ 347.40

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[(aminophenylacetyl)amino]-3-methyl-8-oxo-, monohydrate, [6*R*-[6α,7β(*R**)]]-;
(6*R*,7*R*)-7-[(*R*)-2-Amino-2-phenylacetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid monohydrate [23325-78-2].
Anhydrous [15686-71-2].

DEFINITION

Cephalexin has a potency of NLT 950 µg and NMT 1030 µg of $C_{16}H_{17}N_3O_4S$/mg, calculated on the anhydrous basis.

IDENTIFICATION

• **A. INFRARED ABSORPTION** ⟨197K⟩

Delete the following:

•• **B. ULTRAVIOLET ABSORPTION** ⟨197U⟩
Sample solution: 0.02 mg/mL of Cephalexin in water
Standard solution: 0.02 mg/mL of USP Cephalexin RS in water
Absorptivity: On the anhydrous basis, at peak maxima about 262 nm: 95.0%–104.0% of *Sample solution* to *Standard solution* corrected for potency
Acceptance criteria: Peak maxima and minima at the same wavelengths•$_S$

Add the following:

•• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.•$_S$

Delete the following:

•~~• **C. THIN-LAYER CHROMATOGRAPHY**~~
~~**Standard solution:** 25 mg/mL of USP Cephalexin RS in water with the aid of 0.1 N hydrochloric acid~~
~~**Sample solution:** 25 mg/mL in water, with 0.1 N hydrochloric acid~~
~~**Chromatographic system**~~
~~(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)~~
~~**Mode:** TLC~~
~~**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture~~
~~**Application volume:** 5 µL~~
~~**Developing solvent system:** Ethyl acetate, acetonitrile, glacial acetic acid, and water (21:7:7:9)~~
~~**Analysis**~~
~~**Samples:** *Standard solution* and *Sample solution*~~
~~Allow the spots to dry, and place the plate in a saturated chamber containing the solvent system and lined with filter paper. Develop the chromatogram until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, allow the plate to air-dry, and examine under short-wavelength UV light.~~
~~**Acceptance criteria:** The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.~~•$_S$

ASSAY

Change to read:

• **PROCEDURE**
Mobile phase: 0.985 g/L of sodium-1-pentanesulfonate in a mixture of acetonitrile, methanol, triethylamine, and water (20:10:3:170), adjusted with phosphoric acid to a pH of 3.0 ± 0.1
•$_S$
Standard stock solution: 1 mg/mL of USP Cephalexin RS in water
Standard solution: •0.4 mg/mL of cephalexin in *Mobile phase* from *Standard stock solution*•$_S$
Sample stock solution: 1 mg/mL of Cephalexin in water
Sample solution: •0.4 mg/mL of Cephalexin in *Mobile phase* from *Sample stock solution*•$_S$
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L1 of low acidity
Flow rate: 1.5 mL/min
Injection size: 20 µL
System suitability
Sample: *Standard solution*
•$_S$
Suitability requirements
•$_S$
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the quantity, in µg, of $C_{16}H_{17}N_3O_4S$ per mg of the Cephalexin taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*•$_S$
C_S = concentration of USP Cephalexin RS in the *Standard solution* (mg/mL)
C_U = concentration of Cephalexin in the *Sample solution* (mg/mL)
P = designated content of cephalexin in USP Cephalexin RS (µg/mg)
Acceptance criteria: 950–1030 µg/mg on the anhydrous basis

IMPURITIES

Organic Impurities

• **PROCEDURE 1**
Solution A: Dissolve 1 g of sodium 1-pentanesulfonate in a mixture of 1000 mL of water and 15 mL of triethylamine. Adjust with phosphoric acid to a pH of 2.5 ± 0.1.
Solution B: Dissolve 1 g of sodium 1-pentanesulfonate in a mixture of 300 mL of water and 15 mL of triethylamine. Adjust with phosphoric acid to a pH of 2.5 ± 0.1, and add 350 mL of acetonitrile and 350 mL of methanol.
Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
1	100	0
33.3	0	100
34.3	0	100

Diluent: 18 mg/mL of monobasic potassium phosphate in water
Standard solutions: 0.08 mg/mL and 0.16 mg/mL of $C_{16}H_{17}N_3O_4S$ from USP Cephalexin RS in *Diluent*, taking into account the stated potency of the USP Cephalexin RS

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Sample solution: 5 mg/mL of Cephalexin in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L1 of low acidity
Flow rate: 1 mL/min
Injection size: 20 µL
Analysis
Samples: *Standard solutions* and *Sample solution*
Plot the responses of the cephalexin peaks from the *Standard solutions* versus their concentrations, calculated on the anhydrous basis, in mg/mL, and draw a straight line through the two points and zero. From the line so obtained and the peak responses of the *Sample solution*, determine the concentration, I, in mg/mL, of each cephalexin-related substance of the *Sample solution* other than the cephalexin peak.
Calculate the percentage of each cephalexin-related substance:

$$\text{Result} = I/C \times 100$$

•I = concentration of each cephalexin-related substance in the *Sample solution* as determined from the calibration curve (mg/mL)•$_S$

Acceptance criteria
Individual impurities: NMT 1.0% of any individual cephalexin-related substance
Total impurities: NMT 5.0%
• **PROCEDURE 2: DIMETHYLANILINE ⟨223⟩:** Meets the requirement

SPECIFIC TESTS
• **OPTICAL ROTATION,** *Specific Rotation* ⟨**781S**⟩**:** +149° to +158°
Sample solution: 5 mg/mL, in pH 4.4 neutralized phthalate buffer (See *Reagents, Indicators, and Solutions—Buffer Solutions*)
• **CRYSTALLINITY ⟨695⟩:** Meets the requirements
• **pH ⟨791⟩:** 3.0–5.5, in an aqueous suspension containing 50 mg/mL
• **WATER DETERMINATION,** *Method I* ⟨**921**⟩**:** 4.0%–8.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight containers.
• **USP REFERENCE STANDARDS ⟨11⟩**
USP Cephalexin RS

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