
INTERIM REVISION ANNOUNCEMENT

In this section readers will find the following:

- The list of new USP Reference Standards that have become available
- The list of assays or tests that are adopted but held in abeyance pending availability of required USP Reference Standards
- Newly adopted (official) revisions to the *USP–NF* that become official before the official date of the next *Supplement* or that were not ready for adoption by the closing date for the upcoming *Supplement*. (The official date for these revisions is stated on the next page.)
- Errata

Readers should review this section to determine if they are affected by any of the changes.

Symbols—New text is enclosed in symbols and set off from the current official text as shown in the following example:
•new text•

Where the symbols appear together with no enclosed text, such as ••, it means that text has been deleted and no new text was proposed to replace it. In all revisions, the closing symbol is accompanied by an identifier that indicates the issue of a given *PF* volume.

Errata—Errata are considered to be text, erroneously published in the *USP–NF* or its *Supplements*, that do not accurately reflect the intended official requirements of the Council of Experts. At the end of the *Interim Revision Announcement* section in this publication is a list of errata and corrections to the *USP–NF*. The page number indicates where the item is found in *USP–NF*. Errata lists are updated as necessary in each *Pharmacopeial Forum* and also appear on USP’s website (www.usp.org). Errata lists will be cumulative in future *Supplements*, and the corrected text will appear in the next annual edition of *USP–NF*.

INTERIM REVISION ANNOUNCEMENT	1127
Cephalexin	1131
Cephalexin Capsules	1132
Cephalexin for Oral Suspension	1133
Cephalexin Tablets	1134
Cephalexin Tablets for Oral Suspension	1135
Cephalexin Hydrochloride	1136
Glacial Acetic Acid	1137
Protamine Sulfate	1138
Protamine Sulfate Injection	1138
Protamine Sulfate for Injection	1139
ERRATA LIST FOR <i>USP 32–NF 27</i>	1140

INTERIM REVISION
ANNOUNCEMENT
to *USP 33* and to *NF 28 REISSUE*

*By authority of the United States Pharmacopeial Convention, Inc.
Prepared by the Council of Experts and published by the Board of Trustees*

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Released September 1, 2010

Official October 1, 2010

Inquiries regarding *USP–NF* can be addressed to the USP Executive Secretariat, 12601 Twinbrook Parkway, Rockville, MD 20852, USA (execsec@usp.org).

New USP Reference Standards

The following USP Reference Standards, which were not available when the associated monograph was made official, have since become available. The respective official date of each *USP–NF* standard, test, or assay requiring the use of the following USP Reference Standards is indicated in parentheses after the name of the Reference Standard. Note that the official date is six months after the notice of availability for this Reference Standard was published in *PF*.

USP Powdered *Echinacea Pallida* Extract RS (February 1, 2011)
USP Oleyl Oleate RS (September 1, 2010)

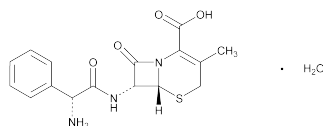
Unavailable First-Time Official USP Reference Standards

The official dates of any *USP–NF* standards, tests, or assays requiring the use of the following new USP Reference Standards are postponed until further notice pending availability of the respective Reference Standards. This listing was updated as of June 15, 2010. Please refer to the current USP Catalog for a more up-to-date availability list. The USP Catalog can be accessed on-line at <http://www.uspcatalog.com>.

USP Acarbose RS
USP Acarbose System Suitability Mixture RS
USP Albumin Human RS
USP Alteplase RS
USP Amifostine RS
USP Amifostine Thiol RS
USP Antithrombin III Human RS
USP Aprotinin RS
USP Aprotinin System Suitability RS
USP Copolymer Polypropylene RS
USP Diethylstilbestrol Diphosphate RS
USP Eucatropine Hydrochloride RS
USP Gonadorelin Hydrochloride RS
USP Hemoglobin RS
USP Maritime Pine Extract RS
USP Menotropins RS
USP Sargramostim RS
USP Sincalide RS
USP Valrubicin Related Compound A RS

MONOGRAPHS (USP)

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, [6R-[6 α , 7 β (R*)]]-;
 (6R,7R)-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 μ g and NMT 1030 μ g 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 \bullet_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. \bullet_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 μ L
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*. \bullet_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

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*. \bullet_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*. \bullet_s

Chromatographic system

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

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm \times 25-cm; packing L1 of low acidity

Flow rate: 1.5 mL/min

Injection size: 20 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the quantity, in μ g, of $C_{16}H_{17}N_3O_4S$ per mg of the Cephalexin taken:

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

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*. \bullet_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 (μ g/mg)

Acceptance criteria: 950–1030 μ g/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

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

Cephalexin Capsules

DEFINITION

Cephalexin Capsules contain the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of cephalexin ($C_{16}H_{17}N_3O_4S$).

IDENTIFICATION

Delete the following:

• A. THIN-LAYER CHROMATOGRAPHY

Standard solution: 3 mg/mL of USP Cephalexin RS in water

Sample solution: 3 mg/mL of cephalexin from Capsules in water and filter

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of binder-free silica gel

Application volume: 10 μL

Pre-developing solvent system: *n*-Hexane and tetradecane (95:5)

Ninhydrin solution: 66.7 mg/mL of ninhydrin in acetone

Developing solvent system: 0.1 M citric acid, 0.1 M dibasic sodium phosphate, and *Ninhydrin solution* (60:40:1.5)

Analysis

Samples: *Standard solution* and *Sample solution*

Allow the solvent front to move the length of the plate in the *Pre-developing solvent system*, remove the plate from the chamber, and allow the solvent to evaporate. On this plate apply 10 μL each of the *Sample solution* and *Standard solution*. Allow the spots to dry, and develop the chromatogram in the *Developing solvent system* 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, dry the plate for 10 min at 110°, and examine the chromatogram.

Acceptance criteria: The R_f value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*._s

Add the following:

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*._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: Equivalent to 1 mg/mL of cephalexin from combined contents of NLT 20 Capsules in water. Sonicate, if necessary, to dissolve the cephalexin. Filter, if necessary, to obtain a clear solution.

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 percentage of C₁₆H₁₇N₃O₄S in the portion of Capsules taken:

$$\bullet \text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = response from the *Sample solution*
r_S = response from the *Standard solution*•_s
C_S = concentration of USP Cephalexin RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of cephalexin in the *Sample solution* (mg/mL)
P = designated content of cephalexin in USP Cephalexin RS (μg/mg)
F = unit conversion factor, 0.001 mg/μg

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS

• **DISSOLUTION** (711)

Medium: Water; 900 mL

Apparatus 1: 100 rpm

Time: 30 min

Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary, to a concentration of about 20 μg/mL.

Standard solution: 20 μg/mL of USP Cephalexin RS in *Medium*

Spectrometric conditions

(See *Spectrophotometry and Light-Scattering* (851).)

Mode: UV

Analytical wavelength: 262 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Tolerances: NLT 80% (Q) of the labeled amount of C₁₆H₁₇N₃O₄S is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

SPECIFIC TESTS

Delete the following:

• **WATER DETERMINATION, Method I** (921): NMT 10.0%•_s

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** (11)
USP Cephalexin RS

Cephalexin for Oral Suspension

DEFINITION

Cephalexin for Oral Suspension is a dry mixture of Cephalexin and one or more suitable buffers, colors, diluents, and flavors. It contains the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of C₁₆H₁₇N₃O₄S per mL when constituted as directed in the labeling.

IDENTIFICATION

Delete the following:

• **A. THIN-LAYER CHROMATOGRAPHY**

Standard solution: 3 mg/mL of USP Cephalexin RS in water

Sample solution: 3 mg/mL of Cephalexin, from Oral Suspension constituted as directed in the labeling and filtered

Ninhydrin solution: 66.7 mg/mL of ninhydrin in acetone

Chromatographic system

Mode: TLC

Adsorbent: 0.25-mm layer of binder-free silica gel

Application volume: 10 μL

Pre-developing solvent: *n*-Hexane and tetradecane (95:5)

Developing solvent: 0.1 M citric acid, 0.1 M dibasic sodium phosphate, and *Ninhydrin solution* (120:80:3)

Analysis

Samples: *Standard solution* and *Sample solution*

Place the plate in *Pre-developing solvent* at a depth of 1 cm and allow the solvent front to move the length of the plate, remove the plate from the chamber, and allow the solvent to evaporate. On this plate apply 10 μL each of the *Sample solution* and the *Standard solution*. Allow the spots to dry, and develop the chromatogram in the *Developing solvent* 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, dry the plate for 10 min at 110°, and examine the chromatogram.

Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*•_s

Add the following:

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

ASSAY

Change to read:

• **PROCEDURE**

Mobile phase: 0.985 g/L of sodium 1-pentanesulfonate in acetonitrile, methanol, triethylamine, and water (20:10:3:170), adjusted with phosphoric acid to a pH of 3.0 ± 0.1

Standard stock solution: 1 mg/mL of USP Cephalexin RS in water

Standard solution: Mix 10.0 mL of *Standard stock solution* with 15.0 mL of *Mobile phase*.

•_s
Sample stock solution: Nominally equivalent to 1 mg/mL of cephalexin from Oral Suspension, constituted as directed in the labeling, freshly mixed and free from air bubbles. Sonicate, if necessary, to assure complete dissolution of the cephalexin. Filter, if necessary, to obtain a clear solution.

Sample solution: Mix 10.0 mL of *Sample stock solution* and 15.0 mL of *Mobile phase*.

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 percentage of C₁₆H₁₇N₃O₄S in each mL of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = cephalexin peak response from the *Sample solution*
r_S = cephalexin peak response from the *Standard solution*

- C_s = concentration of USP Cephalexin RS in the *Standard stock solution* (mg/mL)
 C_u = nominal concentration of cephalexin from the *Sample stock solution* (mg/mL)
 P = designated potency of USP Cephalexin RS ($\mu\text{g}/\text{mg}$)
 F = unit conversion factor, 0.001 mg/ μg
Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** (905) For solid packaged in single-unit containers: meets the requirements
- **DELIVERABLE VOLUME** (698): Meets the requirements

SPECIFIC TESTS**Delete the following:**

- **WATER DETERMINATION, Method I** (921): NMT 2.0% \bullet_s
- **pH** (791): 3.0–6.0, constituted as directed in the labeling

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** (11)
USP Cephalexin RS

Cephalexin Tablets**DEFINITION**

Cephalexin Tablets are prepared from Cephalexin or Cephalexin Hydrochloride. They contain the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of cephalexin ($\text{C}_{16}\text{H}_{17}\text{N}_3\text{O}_4\text{S}$).

IDENTIFICATION**Delete the following:**• **THIN-LAYER CHROMATOGRAPHY**

Standard solution: 3 mg/mL of USP Cephalexin RS in water

Sample solution: 3 mg/mL of cephalexin from powdered Tablets in water and filter

Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of binder-free silica gel

Application volume: 10 μL

Pre-developing solvent system: *n*-Hexane and tetradecane (95:5)

Ninhydrin solution: 66.7 mg/mL of ninhydrin in acetone

Developing solvent system: 0.1 M citric acid, 0.1 M dibasic sodium phosphate, and *Ninhydrin solution* (60:40:1.5)

Analysis

Samples: *Standard solution* and *Sample solution*

Allow the solvent front to move the length of the plate in the *Pre-developing solvent system*, remove the plate from the chamber, and allow the solvent to evaporate. On this plate, apply 10 μL each of the *Sample solution* and *Standard solution*. Allow the spots to dry, and develop the chromatogram in the *Developing solvent system* 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, dry the plate for 10 min at 110°, and examine the chromatogram.

Acceptance criteria: The R_f value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*. \bullet_s

Add the following:

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. \bullet_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). Adjust with phosphoric acid to a pH of 3.0 ± 0.1 .

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*. \bullet_s

Sample stock solution: Equivalent to 1 mg/mL of cephalexin from combined contents of powdered Tablets (NLT 20) in water. Sonicate, if necessary, to assure complete dissolution of the cephalexin. Filter, if necessary, to obtain a clear solution.

Sample solution: 0.4 mg/mL of cephalexin in *Mobile phase* from *Sample stock solution*. \bullet_s

Chromatographic system

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

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm \times 25-cm; packing L1 of low acidity

Flow rate: 1.5 mL/min

Injection size: 20 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $\text{C}_{16}\text{H}_{17}\text{N}_3\text{O}_4\text{S}$ in the portion of Tablets taken:

$$\bullet \text{Result} = (r_u/r_s) \times (C_s/C_u) \times P \times F \times 100$$

r_u = peak response from the *Sample solution*

r_s = peak response from the *Standard solution*. \bullet_s

C_s = concentration of USP Cephalexin RS in the *Standard solution* (mg/mL)

C_u = nominal concentration of cephalexin in the *Sample solution* (mg/mL)

P = designated content of cephalexin in USP Cephalexin RS ($\mu\text{g}/\text{mg}$)

F = unit conversion factor, 0.001 mg/ μg

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS• **DISSOLUTION** (711)**For Cephalexin**

Medium: Water; 900 mL

Apparatus 1: Use 40-mesh cloth and 100 rpm

Time: 30 min

Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute, if necessary, with *Medium* to a concentration that is similar to the *Standard solution*.

Standard solution: 20 $\mu\text{g}/\text{mL}$ of USP Cephalexin RS in *Medium*

Spectrometric conditions

(See *Spectrophotometry and Light-Scattering* (851).)

Mode: UV
Analytical wavelength: 262 nm

Analysis

Samples: *Standard solution* and *Sample solution*
Tolerances: NLT 80% (Q) of the labeled amount of $C_{16}H_{17}N_3O_4S$ is dissolved.

For Cephalexin hydrochloride

Medium, Sample solution, Standard solution, Spectrometric conditions, and Analysis: Proceed as directed For *Cephalexin*.

Apparatus 1: Use 10-mesh cloth and 150 rpm

Time: 45 min

Tolerances: NLT 75% (Q) of the labeled amount of $C_{16}H_{17}N_3O_4S$ is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

SPECIFIC TESTS

Delete the following:

- **WATER DETERMINATION, Method I (921):** NMT 9.0% where Tablets contain Cephalexin; NMT 8.0% where Tablets contain Cephalexin Hydrochloride^{•5}

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** The label states whether the Tablets contain Cephalexin or Cephalexin Hydrochloride.
- **USP REFERENCE STANDARDS (11)**
USP Cephalexin RS

Cephalexin Tablets for Oral Suspension

DEFINITION

Cephalexin Tablets for Oral Suspension contain NLT 90.0% and NMT 110.0% of the labeled amount of cephalexin ($C_{16}H_{17}N_3O_4S$).

IDENTIFICATION

Delete the following:

- **A. THIN-LAYER CHROMATOGRAPHY**

Standard solution: 3 mg/mL of USP Cephalexin RS in water

Sample solution: 3 mg/mL of cephalexin from powdered Tablets for Oral Suspension in water and filter

Chromatographic system

(See *Chromatography (621), Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of binder-free silica gel

Application volume: 10 μ L

Pre-developing solvent system: *n*-Hexane and tetradecane (95:5)

Ninhydrin solution: 66.7 mg/mL of ninhydrin in acetone

Developing solvent system: 0.1 M citric acid, 0.1 M dibasic sodium phosphate, and *Ninhydrin solution* (60:40:1.5)

Analysis

Samples: *Standard solution* and *Sample solution*

Allow the solvent front to move the length of the plate in the *Pre-developing solvent system*, remove the plate from the chamber, and allow the solvent to evaporate. On this plate apply 10 μ L each of the *Standard solution* and *Sample solution*. Allow the spots to dry, and develop the chromatogram in the *Developing solvent system* 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, dry the plate for 10 min at 110°, and examine the chromatogram.

Acceptance criteria: The R_f value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.^{•5}

Add the following:

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.^{•5}

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

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*.^{•5}

Sample stock solution: Nominally equivalent to 1 mg/mL of cephalexin from combined contents of NLT 20 powdered Tablets for Oral Suspension in water. Pass a portion of the solution through a filter having a 1- μ m or finer porosity.

Sample solution: 0.4 mg/mL of cephalexin in *Mobile phase* from *Sample stock solution*.^{•5}

Chromatographic system

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

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm \times 25-cm; packing L1 of low acidity

Flow rate: 1.5 mL/min

Injection size: 20 μ L

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{16}H_{17}N_3O_4S$ in each Tablet for Oral Suspension:

$$\bullet \text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

r_U = response from the *Sample solution*

r_S = response from the *Standard solution*.^{•5}

C_S = concentration of USP Cephalexin RS in the *Sample stock solution* (mg/mL)

C_U = nominal concentration of cephalexin in the *Sample stock solution* (mg/mL)

P = designated content of cephalexin in USP Cephalexin RS (μ g/mg)

F = unit conversion factor, 0.001 mg/ μ g

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DISINTEGRATION (701):** Tablets for Oral Suspension disintegrate in 3 min, using water at $20 \pm 5^\circ$.

- **DISSOLUTION (711)**

Medium: Water; 900 mL

Apparatus 1: Use 40-mesh cloth and 100 rpm

Time: 30 min

Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary, to a concentration of about 20 μ g/mL.

Standard solution: 20 μ g/mL of USP Cephalexin RS in *Medium*

Spectrometric conditions

(See *Spectrophotometry and Light-Scattering (851)*.)

Mode: UV

Analytical wavelength: 262 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Tolerances: NLT 80% (Q) of the labeled amount of $C_{16}H_{17}N_3O_4S$ is dissolved.

- **DISPERSION FINENESS:** Place 2 Tablets for Oral Suspension in 100 mL of water, and stir until completely dispersed. A smooth dispersion is obtained that passes through a No. 25 sieve.
- **UNIFORMITY OF DOSAGE UNITS (905):** Meets the requirements

SPECIFIC TESTS

Delete the following:

- **WATER DETERMINATION, Method I (921):** NMT 9.0%_s

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**
USP Cephalexin RS

Cephalexin Hydrochloride

$C_{16}H_{17}N_3O_4S \cdot HCl \cdot H_2O$ 401.87

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[(aminophenylacetyl)amino]-3-methyl-8-oxo-, monohydrochloride, monohydrate, [6R-[6 α ,7 β (R*)]]-;

(6R,7R)-7-[(2R)-2-Amino-2-phenylacetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, monohydrochloride, monohydrate;

7-(D-2-Amino-2-phenylacetamido)-3-methyl-3-cephem-4-carboxylic acid hydrochloride monohydrate [105879-42-3].

DEFINITION

Cephalexin Hydrochloride contains the equivalent of NLT 800 μ g and NMT 880 μ g of cephalexin ($C_{16}H_{17}N_3O_4S$) per mg.

IDENTIFICATION

Delete the following:

• A. 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 the aid of 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 μ L

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, 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

Add the following:

- **A.** 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:

• B. PROCEDURE

Sample solution: 0.02 mg/mL of cephalexin in water

Analysis: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Cephalexin RS, concomitantly measured._s

Change to read:

- **B._s IDENTIFICATION TESTS—GENERAL, Chloride (191):** 10 mg/mL meets the requirements

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: 1.15 mg/mL of Cephalexin Hydrochloride 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 \times 25-cm; packing L1 of low acidity

Flow rate: 1.5 mL/min

Injection size: 20 μ L

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 μ g, of $C_{16}H_{17}N_3O_4S$ in each mg of Cephalexin Hydrochloride taken:

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

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 stock solution* (mg/mL)

C_U = concentration of Cephalexin Hydrochloride from the *Sample stock solution* (mg/mL)

P = designated content of cephalexin in USP Cephalexin RS (μ g/mg)

Acceptance criteria: 800–880 µg/mg

IMPURITIES

Organic Impurities

• **PROCEDURE 1**

Solution A: 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: 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 C₁₆H₁₇N₃O₄S from USP Cephalexin RS in *Diluent*, taking into account the stated potency of the USP Cephalexin RS

Sample solution: 6 mg/mL of Cephalexin Hydrochloride 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 of 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 from the *Sample solution* other than the cephalexin peak.

Calculate the percentage of each cephalexin-related substance represented by each peak of the *Sample solution*, other than the cephalexin peak.

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

I = concentration of each cephalexin-related substance other than cephalexin in the *Sample solution* (mg/mL)

C = concentration mg/mL of cephalexin from the *Sample solution*

Acceptance criteria

Individual impurities: NMT 1.0% of any individual cephalexin-related substance is found.

Total impurities: NMT 5.0%
• **PROCEDURE 2: DIMETHYLANILINE (223):** Meets the requirement

SPECIFIC TESTS

- **CRYSTALLINITY (695):** Meets the requirements
- **PH (791):** 1.5–3.0, in a solution containing 10 mg/mL
- **WATER DETERMINATION, Method I (921):** 3.0%–6.5%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS (11)**
USP Cephalexin RS

Glacial Acetic Acid



C₂H₄O₂ 60.05
Acetic acid [64-19-7].

DEFINITION

Glacial Acetic Acid contains NLT 99.5% and NMT 100.5%, by weight, of C₂H₄O₂.

IDENTIFICATION

Change to read:

- **IDENTIFICATION TESTS—GENERAL, Acetate (191):** Meets the requirements

Sample solution • (for lanthanum nitrate test): •_s Glacial Acetic Acid and water • (1:100) •_s

ASSAY

• **PROCEDURE**

Sample solution: Measure 2 mL of Glacial Acetic Acid into a glass-stoppered flask, previously tared while containing about 20 mL of water, and weigh again to obtain the weight of the substance under assay.

Analysis: Add 20 mL of water, then add phenolphthalein TS. Titrate with 1 N sodium hydroxide VS. Each mL of 1 N sodium hydroxide is equivalent to 60.05 mg of C₂H₄O₂.

Acceptance criteria: 99.5%–100.5%

IMPURITIES

Inorganic Impurities

- **LIMIT OF NONVOLATILE RESIDUE:** Evaporate 20 mL in a tared dish, and dry at 105° for 1 h: the weight of the residue does not exceed 1.0 mg.
- **HEAVY METALS (231):** NMT 5 ppm
Sample solution: To the residue obtained in the test for *Limit of Nonvolatile Residue* add 8 mL of 0.1 N hydrochloric acid, warm gently until solution is complete, dilute with water to 100 mL, and use 20 mL.
- **CHLORIDE AND SULFATE, Chloride (221)**
Sample solution: Dilute 1.0 mL with 20 mL of water.
Analysis: Add 5 drops of silver nitrate TS.
Acceptance criteria: No opalescence is produced.
- **CHLORIDE AND SULFATE, Sulfate (221)**
Sample solution: Dilute 1.0 mL with 10 mL of water.
Analysis: Add 1 mL of barium chloride TS.
Acceptance criteria: No turbidity is produced.

Organic Impurities

- **PROCEDURE: READILY OXIDIZABLE SUBSTANCES**
Sample solution: Dilute 2.0 mL in a glass-stoppered vessel with 10 mL of water.
Analysis: Add 0.10 mL of 0.10 N potassium permanganate.
Acceptance criteria: The pink color is not changed to brown within 2 h.

SPECIFIC TESTS

- **CONGEALING TEMPERATURE** (651): NLT 15.6°

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at room temperature.

Protamine Sulfate**DEFINITION**

Protamine Sulfate is a purified mixture of simple protein principles obtained from the sperm or testes of suitable species of fish, which has the property of neutralizing heparin. Each mg of Protamine Sulfate, calculated on the dried basis, neutralizes NLT 100 USP Heparin Units.

ASSAY**Change to read:**• **PROCEDURE**

• **Sample solution A:** 0.15 mg/mL of Protamine Sulfate in water

Sample solution B: Dilute 2.0 mL of *Sample solution A* with water to 3.0 mL.

Sample solution C: Dilute 1.0 mL of *Sample solution A* with water to 3.0 mL.

Titrant: USP Heparin Sodium for Assays RS in water (about 80–120 USP Heparin Units/mL)

Analysis: [NOTE—Titrate each *Sample solution* in duplicate.] Transfer a volume of the *Sample solution* to the analytical cell of a suitable colorimeter, and set the apparatus for measurement at a suitable wavelength (none is critical) in the visible range. Add *Titrant* in small volumes until there is a sharp increase in the absorbance, and note the volume of *Titrant* added. Perform the entire *Assay* in triplicate for a total of 18 determinations.

Calculate the number of USP Heparin Units in the volume of *Titrant* added at the endpoint per mg of Protamine Sulfate. Calculate the USP Heparin Units neutralized per mg of Protamine Sulfate taken:

$$\text{Result} = (V_T \times C_T) / (V_S \times C_S)$$

V_T = volume of *Titrant* added (mL)

C_T = concentration of *Titrant* (USP Heparin Units/mL)

V_S = volume of the *Sample solution* (mL)

C_S = concentration of Protamine Sulfate (mg/mL)

Calculate the potency of the Protamine Sulfate as the average of the 18 values. Calculate the 3 standard deviations for the results obtained with each of the *Sample solutions*. Calculate the 3 standard deviations for the results obtained with each of the 3 independent assays. The *Assay* is valid if each of the 6 standard deviations is NMT 5% of the average result.

Acceptance criteria: Each mg of Protamine Sulfate neutralizes NLT 100 USP Heparin Units, on the dried basis.●₅

OTHER COMPONENTS

- **NITROGEN DETERMINATION, Method II** (461)
Acceptance criteria: 22.5%–25.5% of N, on the dried basis.

SPECIFIC TESTS

- **LOSS ON DRYING** (731): Dry a sample at 105° for 3 h: it loses NMT 5% of its weight.

Change to read:• **ULTRAVIOLET ABSORBANCE**

Sample solution: 1.0% solution of Protamine Sulfate ● in water●₅

Spectrometric conditions

(See *Spectrophotometry and Light-Scattering* (851).)

Mode: UV

Wavelength range: Between 260 and 280 nm

Blank: Water

- Acceptance criteria:** The difference in absorbance between 260 and 280 nm of the *Sample solution* against the *Blank* is NMT 0.1.

• **SULFATE**

Sample: 150 mg

Analysis: Dissolve the *Sample* in 75 mL of water, add 5 mL of 3 N hydrochloric acid, heat to boiling, and while maintaining at the boiling point, slowly add 10 mL of barium chloride TS. Cover the vessel, and allow the mixture to stand on a steam bath for 1 h. Filter, wash the precipitate with several portions of hot water, dry, and ignite to constant weight. The weight of the barium sulfate, multiplied by 0.4117, represents the weight of sulfate in the portion of Protamine Sulfate taken.

Acceptance criteria: 16%–22% on the dried basis

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers in a refrigerator.

Change to read:• **USP REFERENCE STANDARDS** (11)

USP Heparin Sodium ● for Assays●₅ RS

Protamine Sulfate Injection**DEFINITION****Change to read:**

Protamine Sulfate Injection is a sterile, isotonic solution of Protamine Sulfate. ● Each mg of Protamine Sulfate, used in the manufacture of the Injection, neutralizes NLT 100 USP Heparin Units, calculated on the dried basis.●₅ It contains NLT 90.0% and NMT 120.0% of the labeled amount of protamine sulfate.

IDENTIFICATION

- **IDENTIFICATION TESTS—GENERAL, Sulfate** (191)

ASSAY**Change to read:**• **PROCEDURE**

Sample solution: ● 0.15 mg/mL●₅ of protamine sulfate in Water for Injection from a measured volume of Injection

• **Analysis:** [NOTE—Titrate the *Sample solution* in duplicate.] Transfer the same volume of the *Sample solution* to the analytical cell as used in the *Assay* for the drug substance. Proceed as directed in the *Assay* under *Protamine Sulfate*, using the same concentration of *Titrant* and the same wavelength as used in the *Assay* for the drug substance. The concentration of the *Sample solution* of the drug substance should also be 0.15 mg/mL. Perform the entire *Assay* in triplicate, and calculate the average of the triplicate determinations. The percentage of the label claim is given as follows:

$$\text{Result} = (v/V) \times 100$$

v = volume of *Titrant* added to the Injection *Sample solution* (mL)

V = volume of *Titrant* added to the drug substance *Sample solution* (mL)

●₅

Acceptance criteria: 90.0%–120.0%

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST (85):** It contains NMT 7.0 USP Endotoxin Units/mg of protamine sulfate.
- **OTHER REQUIREMENTS:** It meets the requirements under *Injections (1)*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I glass. Store at controlled room temperature.
- **LABELING:** Label it to indicate the approximate neutralization capacity in USP Heparin Units.

Change to read:

- **USP REFERENCE STANDARDS (11)**
USP Endotoxin RS
USP Heparin Sodium ^s for Assays ^s RS

Protamine Sulfate for Injection

DEFINITION

Change to read:

Protamine Sulfate for Injection is a sterile mixture of Protamine Sulfate with one or more suitable, dry diluents. Each mg of Protamine Sulfate, used in the manufacture of the Protamine Sulfate for Injection, neutralizes NLT 100 USP Heparin Units, calculated on the dried basis. It contains NLT 90.0% and NMT 120.0% of the labeled amount of protamine sulfate.

ASSAY

Change to read:

- **PROCEDURE**
Sample solution: 0.15 mg/mL of protamine sulfate in Water for Injection. Dissolve from the contents of 1 container of Protamine Sulfate for Injection.
Analysis: [NOTE—Titrate the *Sample solution* in duplicate.] Transfer the same volume of the *Sample solution* to the analyti-

cal cell as used in the *Assay* for the drug substance. Proceed as directed in the *Assay* under *Protamine Sulfate*, using the same concentration of *Titrant* and the same wavelength as used in the *Assay* for the drug substance. The concentration of the *Sample solution* of the drug substance should also be 0.15 mg/mL. Perform the entire *Assay* in triplicate, and calculate the average of the triplicate determinations. The percentage of the label claim is given as follows:

$$\text{Result} = (v/V) \times 100$$

- v = volume of *Titrant* added to the Protamine Sulfate for Injection *Sample solution* (mL)
- V = volume of *Titrant* added to the drug substance *Sample solution* (mL)

Acceptance criteria: 90.0%–120.0%

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS (905):** Meets the requirements

SPECIFIC TESTS

- **INJECTIONS, *Constituted Solutions (1)*:** At the time of use, it meets the requirements.
- **BACTERIAL ENDOTOXINS TEST (85):** It contains NMT 7.0 USP Endotoxin Units/mg of protamine sulfate.
- **STERILITY TESTS (71):** Both it and the accompanying solvent meet the requirements
- **PH AND CLARITY OF SOLUTION:** Dissolve it in the solvent recommended in the labeling: the pH of the solution is 6.5–7.5, and the solution is clear.
- **OTHER REQUIREMENTS:** Both it and the accompanying solvent meet the requirements for *Injections (1)*, *Labeling*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve as described under *Injections (1)*, *Containers for Sterile Solids*. Preserve the accompanying solvent in single-dose or in multiple-dose containers, preferably of Type I glass.
- **LABELING:** Label it to indicate the approximate neutralization capacity in USP Heparin Units.

Change to read:

- **USP REFERENCE STANDARDS (11)**
USP Endotoxin RS
USP Heparin Sodium ^s for Assays ^s RS