कर्नाटका एन्टिबायोटिक्स एण्ड फार्मास्युटिकल्स लिमिटेड
(भारत सरकार का उद्यम)
KARNATAKA ANTIBIOTICS & PHARMACEUTICALS LIMITED
(A Government of India Enterprise)

ENQUIRY REF. No.	KAPL/QAD/020/1385
DATE	23/09/2024
DUE DATE	26/09/2024 (13.00HRS)

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02	QSPHPL225	30CMX4MM C18 SILICAGEL 10 MICRON	NOS	03

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Thanking you,

Yours faithfully,
For KARNATAKA ANTIBIOTICS
& PHARMACEUTICALS LIMITED

YUVARAJA M
DEPUTY MANAGER PURCHASE DEPT
MOB:9945317873

nu Sep 19 2024, 10:52:54 am
Shashidhar B
urrently Official on 19-Sep-2024
Date: Official as of 01-May-2018
ment Type: USP
eld: GUID-B552EE29-D951-4EE0-9CBB-8C83BFF4BF8C_3_en-US
oI: https://doi.org/10.31003/USPNF_M4528_03_01
DOI Ref: q3uqe

Ampicillin and Sulbactam for Injection

» Ampicillin and Sulbactam for Injection is a sterile, dry mixture of Ampicillin Sodium and Sulbactam Sodium. It contains the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amounts of ampicillin ($C_{16}H_{19}N_3O_4S$) and sulbactam ($C_8H_{11}NO_5S$), the labeled amounts representing proportions of ampicillin to sulbactam of 2:1. It contains not less than 563 µg of ampicillin and 280 µg of sulbactam per mg, calculated on the anhydrous basis.

Packaging and storage—Preserve as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*.

USP Reference Standards ⟨11⟩—
USP Ampicillin RS
USP Sulbactam RS

Constituted solution—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions.*

Identification—The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Bacterial Endotoxins Test ⟨85⟩ —It contains not more than 0.17 USP Endotoxin Unit in a portion equivalent to 1 mg of a mixture of ampicillin and sulbactam (0.67 and 0.33 mg, respectively).

Sterility Tests ⟨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 8.0 and 10.0, in a solution containing 10 mg of ampicillin and 5 mg of sulbactam per mL.

Water Determination, *Method I* ⟨921⟩ **:** not more than 2.0%.

Particulate Matter in Injections ⟨788⟩ **:** meets the requirements for small-volume injections.

Other requirements—It meets the requirements for *Uniformity of Dosage Units* ⟨905⟩ and for *Labeling* ⟨7⟩, *Labels and Labeling for Injectable Products*.

Assay—

0.005 M Tetrabutylammonium hydroxide—Dilute 6.6 mL of a 40% solution of tetrabutylammonium hydroxide with water to obtain 1800 mL of solution. Adjust with 1 M phosphoric acid to a pH of 5.0 ± 0.1, dilute with water to 2000 mL, and mix.

Mobile phase—Prepare a filtered and degassed mixture of *0.005 M Tetrabutylammonium hydroxide* and acetonitrile (1650:350). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Quantitatively dissolve accurately weighed quantities of USP Ampicillin RS and USP Sulbactam RS in *Mobile phase* to obtain a solution having known concentrations of about 0.6 mg of ampicillin per mL and 0.3 mg of sulbactam per mL. [Note—Inject this solution promptly.]

Resolution solution—Prepare a solution of USP Sulbactam RS in 0.01 N sodium hydroxide containing 0.3 mg per mL, and allow to stand for 30 minutes. Adjust with phosphoric acid to a pH of 5.0 ± 0.1. Transfer 5 mL of the solution to a 25-mL volumetric flask, add 4.25 mL of acetonitrile, dilute with *0.005 M Tetrabutylammonium hydroxide* to volume, and mix. Transfer 1 mL of this solution to a second 25-mL volumetric flask, add 15 mg of USP Ampicillin RS, dilute with *Mobile phase* to volume, and mix. [Note—Inject this solution promptly.]

Assay preparation 1—Mix the contents of a container of Ampicillin and Sulbactam for Injection. Quantitatively dissolve an accurately weighed portion of the powder in *Mobile phase* to obtain a solution having a concentration of about 1 mg of the powder per mL. [Note—Inject this solution promptly.]

Assay preparation 2 (where it is represented as being in a single-dose container)— Constitute a container of Ampicillin and Sulbactam for Injection with a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw the total withdrawable contents from the container, using a suitable hypodermic needle and syringe, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution containing about 0.6 mg of ampicillin per mL and 0.3 mg of sulbactam per mL. [Note—Inject this solution promptly.]

Assay preparation 3 (where the label states the quantities of ampicillin and sulbactam in a given volume of constituted solution)— Constitute a container of Ampicillin and Sulbactam for Injection with 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, and stepwise if necessary, with *Mobile phase* to obtain a solution containing about 0.6 mg of ampicillin per mL and 0.3 mg of sulbactam per mL. [Note—Inject this solution promptly.]

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 230-nm detector and a 4-mm × 30-cm column containing packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Resolution solution*, and record the responses as directed for *Procedure:* the relative retention times are about 0.7 for ampicillin and 1.0 for sulbactam alkaline degradation product; and the resolution, *R*, between ampicillin and sulbactam alkaline degradation product is not less than 4.0. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure:* the relative retention times are about 0.35 for ampicillin and 1.0 for sulbactam; the column efficiency determined from the sulbactam peak is not less than 3500 theoretical plates; the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the appropriate *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantities, in µg, of ampicillin ($C_{16}H_{19}N_3O_4S$) and of sulbactam ($C_8H_{11}NO_5S$) in the portion of Ampicillin and Sulbactam for Injection taken by the same formula:

$$(C_S P/C_U)(r_U/r_S)$$

in which C_S is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation; P* is the assigned content, in µg per mg, of the appropriate USP Reference Standard; C_U is the concentration, in mg per mL, of Ampicillin and Sulbactam for Injection in *Assay preparation 1*, based on the weight, in mg, of powder removed from the container and the extent of dilution; and r_U and r_S are the peak areas for the appropriate analyte obtained from *Assay preparation 1* and the *Standard preparation*, respectively. Calculate the quantities of ampicillin ($C_{16}H_{19}N_3O_4S$) and of sulbactam ($C_8H_{11}NO_5S$) withdrawn from the container, or in the volume of constituted solution taken by the same formula:

$$(L/D)(C_S P)(r_U/r_S)$$

in which *L* is the labeled quantity, in mg, of ampicillin or sulbactam, as appropriate, in the container or in the volume of constituted solution taken; *D* is the concentration, in mg per mL, of ampicillin or sulbactam in *Assay preparation 2* or *Assay preparation 3*, on the basis of the labeled quantity, in mg, of ampicillin or sulbactam, as appropriate, in the container and the extent of dilution; r_U and r_S are the peak areas for the appropriate analyte obtained from *Assay preparation 2* or *Assay preparation 3* and the *Standard preparation*, respectively; and the other terms are as defined above.

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

Topic/Question	Contact	Expert Committee
AMPICILLIN AND SULBACTAM FOR INJECTION	Documentary Standards Support	SM12020 Small Molecules 1

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:
Pharmacopeial Forum: Volume No. 44(1)

Page Information:

USP43-NF38 - 328
USP42-NF37 - 323
USP41-NF36 - 305

Current DocID: GUID-B552EE29-D951-4EE0-9CBB-8C83BFF4BF8C_3_en-US
Previous DocID: GUID-B552EE29-D951-4EE0-9CBB-8C83BFF4BF8C_1_en-US
DOI: https://doi.org/10.31003/USPNF_M4528_03_01
DOI ref: q3uqe

Ampicillin Sodium

$C_{16}H_{18}N_3NaO_4S$ 371.39

4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, [6-(aminophenylacetyl)amino]-3,3-dimethyl-7-oxo-, monosodium salt, [2*S*-[2α,5α,6β(*S**)]]-;

Monosodium *D*-(−)-6-(2-amino-2-phenylacetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate [69-52-3]; UNII: JFN36L5S8K.

DEFINITION

Ampicillin Sodium has a potency equivalent to NLT 845 µg and NMT 988 µg of ampicillin ($C_{16}H_{19}N_3O_4S$) per mg, calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A.** ▲**Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197M**▲ (CN 1-May-2020)
- **B. Identification Tests—General,** *Sodium* ⟨191⟩

ASSAY

- **Procedure**

Diluent: Water, 1 M monobasic potassium phosphate, and 1 N acetic acid (989:10:1)

Mobile phase: Acetonitrile, water, 1 M monobasic potassium phosphate, and 1 N acetic acid (80:909:10:1)

Standard solution: 1 mg/mL of USP Ampicillin RS in *Diluent* using shaking and sonication, if necessary, to dissolve. Use this solution promptly after preparation.

System suitability solution: 0.12 mg/mL of caffeine in *Standard solution*

Sample solution: [Note—Ampicillin Sodium is hygroscopic. Minimize exposure to the atmosphere, and weigh promptly.] Equivalent to 1 mg/mL of anhydrous ampicillin in *Diluent*.[Note—Use this solution promptly after preparation.]

Chromatographic system

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

Mode: LC

Detector: UV 254 nm

Column

Pre-column: 4-mm × 5-cm; 5- to 10-µm packing L1

Analytical column: 4-mm × 30-cm; 5- to 10-µm packing L1

Flow rate: 2 mL/min

Injection size: 20 µL

System suitability

Samples: *Standard solution* and *System suitability solution*

[Note—The relative retention times for ampicillin and caffeine are 0.5 and 1.0, respectively, *System suitability solution*.]

Suitability requirements

Resolution: NLT 2.0 between the caffeine and the ampicillin peaks, *System suitability solution*

Tailing factor: NMT 1.4, *Standard solution*

Capacity factor: NMT 2.5, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the quantity, in µg, of $C_{16}H_{19}N_3O_4S$ in each mg of Ampicillin Sodium 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*

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

C_U = nominal concentration of Ampicillin Sodium in the *Sample solution* (mg/mL)

P = potency of USP Ampicillin RS (µg/mg)

Acceptance criteria: 845–988 µg/mg on the anhydrous basis

IMPURITIES

ORGANIC IMPURITIES

- **PROCEDURE 1: LIMIT OF METHYLENE CHLORIDE**

Internal standard solution: 2.1 mg/mL of dioxane in dimethyl sulfoxide

Standard solution: 0.33 mg/mL of methylene chloride in *Internal standard solution*

Sample solution: 166.7 mg/mL of Ampicillin Sodium in *Internal standard solution*

Chromatographic system

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

Mode: GC

Detector: Flame ionization

Column: 1.8-m × 4-mm glass column packed with a 10% phase G39 on unsilanized support S1A

Temperature

Column: 65°

Injector: 100°

Detector block: 260°

Carrier gas: Nitrogen

Flow rate: 60 mL/min

Injection size: 1 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for methylene chloride and dioxane are 0.5 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 4 between methylene chloride and dioxane

Relative standard deviation: NMT 5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of methylene chloride in the portion of Ampicillin Sodium taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of methylene chloride to dioxane from the *Sample solution*

R_S = peak response ratio of methylene chloride to dioxane from the *Standard solution*

C_S = concentration of methylene chloride in the *Standard solution* (mg/mL)

C_U = nominal concentration of Ampicillin Sodium in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.2%.

- **PROCEDURE 2: DIMETHYLANILINE ⟨223⟩:** Meets the requirement

SPECIFIC TESTS

- **CRYSTALLINITY ⟨695⟩:** Meets the requirements. [NOTE—Ampicillin Sodium in the freeze-dried form is exempt from this requirement.]
- **pH ⟨791⟩:** 8.0–10.0

Sample solution: 10.0 mg/mL of ampicillin

- **WATER DETEMINATION, *Method I*⟨921⟩:** NMT 2.0%
- **STERILITY TESTS ⟨71⟩:** Where the label states that Ampicillin Sodium is sterile, it meets the requirements.
- **BACTERIAL ENDOTOXINS TEST ⟨85⟩:** Where the label states that Ampicillin Sodium is sterile or the label states that Ampicillin Sodium must be subjected to further processing during the processing of injectable dosage forms, it contains NMT 0.15 USP Endotoxin Unit/mg of ampicillin.

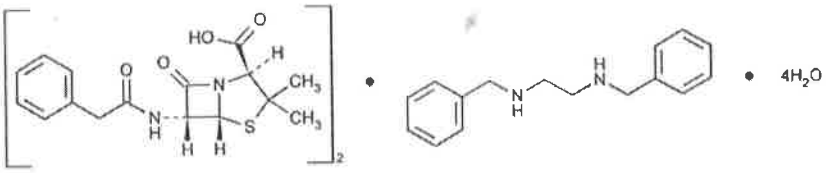
ADDITIONAL REQUIREMENTS

- **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 Ampicillin RS

USP Ampicillin Sodium RS

Penicillin G Benzathine



$(C_{16}H_{18}N_2O_4S)_2 \cdot C_{16}H_{20}N_2 \cdot 4H_2O$ 981.18

4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 3,3-dimethyl-7-oxo-6-[(phenylacetyl)amino-], 2[*S*-(2α,5α,6β)]-, compd. with *N*,*N*′-bis(phenylmethyl)-1,2-ethanediamine (2:1), tetrahydrate.

(2*S*,5*R*,6*R*)-3,3-Dimethyl-7-oxo-6-(2-phenylacetamido)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid compound with *N*,*N*′-dibenzylethylenediamine (2:1), tetrahydrate [41372-02-5]; UNII: RIT82F58GK.

Anhydrous 909.15 [1538-09-6]; UNII: SSZ1S4I0US.

» Penicillin G Benzathine has a potency of not less than 1090 Penicillin G Units and not more than 1272 Penicillin G Units per mg.

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 Penicillin G Benzathine RS
USP Penicillin G Potassium RS

Identification, *Ultraviolet Absorption* (197U)—

Solution: 500 µg per mL.

Medium: methanol.

Absorptivity at 263 nm is between 85.0% and 110.0% of that of USP Penicillin G Benzathine RS.

Crystallinity (695): meets the requirements.

Bacterial Endotoxins Test (85) —Where the label states that Penicillin G Benzathine is sterile or that it must be subjected to further processing during the preparation of injectable dosage forms it contains not more than 0.01 USP Endotoxin Unit per 100 Penicillin G Units.

Sterility Tests (71) —Where the label states that Penicillin G Benzathine is sterile it meets the requirements when tested as directed in the section *Direct Inoculation of the Culture Medium* under *Test for Sterility of the Product to be Examined*, except to use Fluid Thioglycollate Medium and Soybean–Casein Digest Medium containing polysorbate 80 solution (1 in 200) and an amount of sterile penicillinase sufficient to inactivate the penicillin G in each tube, and to shake the vessels once daily.

pH (791): between 4.0 and 6.5, in a solution prepared by dissolving 50 mg in 50 mL of dehydrated alcohol, adding 50 mL of water, and mixing.

Water Determination, *Method I* (921): between 5.0% and 8.0%.

Benzathine content—To about 1 g of Pencillin G Benzathine, accurately weighed, add 30 mL of a saturated solution of sodium chloride and 10 mL of 5 N sodium hydroxide, and extract with four 50-mL portions of ether. Wash the combined ether extracts with three 10-mL portions of water. Extract the combined water washings with 25 mL of ether, and add the ether extract to the water-washed combined ether extracts. Evaporate this combined ether solution to a volume of about 5 mL, add 2 mL of dehydrated alcohol, and evaporate to dryness. Dissolve the residue in 50 mL of glacial acetic acid, add 1 mL of *p*-naphtholbenzein TS, and titrate with 0.1 N perchloric acid VS to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 12.02 mg of benzathine ($C_{16}H_{20}N_2$): between 24.0% and 27.0% of benzathine in Penicillin G Benzathine, calculated on the anhydrous basis, is found.

Assay—

0.05 M phosphate buffer, pH 6.0—Dissolve 6.8 g of monobasic potassium phosphate in 900 mL of water, adjust with 1 N sodium hydroxide to a pH of 6.0, dilute with water to 1000 mL, and mix.

Mobile phase—Prepare a mixture of *0.05 M phosphate buffer, pH 6.0* and acetonitrile (4:1), pass through a membrane filter having a 5-µm or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Transfer about 40 mg of USP Penicillin G Potassium RS, accurately weighed, to a 50-mL volumetric flask, add 10 mL of acetonitrile and 5 mL of methanol, and swirl to dissolve. Without delay, dilute with *0.05 M phosphate buffer, pH 6.0* to volume, and mix.

System suitability preparation—Prepare a solution of penicillin V potassium in *Mobile phase* containing about 1 mg per mL. Mix equal volumes of this solution and the *Standard preparation*.

Assay preparation—Transfer about 53 mg of Penicillin G Benzathine, accurately weighed, to a 50-mL volumetric flask, add 10 mL of

acetonitrile and 5 mL of methanol, and swirl to dissolve. Without delay, dilute with *0.05 M phosphate buffer, pH 6.0* to volume, and mix.
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 225-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation* and the *System suitability preparation*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.7 for penicillin G and 1.0 for penicillin V; the resolution, *R,* between penicillin G and penicillin V is not less than 2.0; the column efficiency determined from the analyte peak is not less than 600 theoretical plates; and the relative standard deviation for replicate injections of the *Standard preparation* is not more than 1.0%.
Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the potency, in Penicillin G Units per mg, of the Penicillin G Benzathine taken by the formula:

$$50(CP/W)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Penicillin G Potassium RS in the *Standard preparation;* P is the stated potency, in Penicillin G Units per mg, of USP Penicillin G Potassium RS; W is the quantity, in mg, of Penicillin G Benzathine taken to prepare the *Assay preparation;* and r_U and r_S are the penicillin G peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

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

Topic/Question	Contact	Expert Committee
PENICILLIN G BENZATHINE	Christine Hiemer Scientific Liaison	SM12020 Small Molecules 1

Chromatographic Database Information: Chromatographic Database

Page Information:

USP42-NF37 - 3405
USP41-NF36 - 3194
USP40-NF35 - 5598

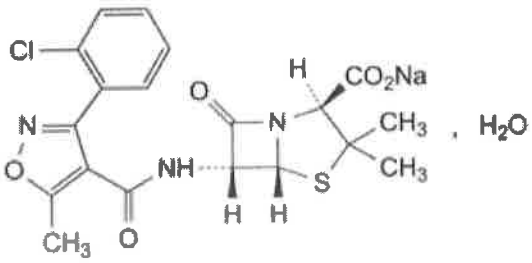
Current DocID: GUID-DF853AE1-66A0-4B1C-A3CC-3629C5552CD6_3_en-US
Previous DocID: GUID-DF853AE1-66A0-4B1C-A3CC-3629C5552CD6_1_en-US

Entries in this edition will be effective from 01 July 2024

Cloxacillin Sodium

General Notices

(Ph. Eur. monograph 0661)



$C_{19}H_{17}ClN_3NaO_5S,H_2O$ 475.9 *7081-44-9*

Action and use

Penicillin antibacterial.

Ph Eur

DEFINITION

Sodium (2*S*,5*R*,6*R*)-6-[[[3-(2-chlorophenyl)-5-methyl-1,2-oxazol-4-yl]carbonyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate monohydrate.

Semi-synthetic product derived from a fermentation product.

Content

95.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or almost white, hygroscopic, crystalline powder.

Solubility

Freely soluble in water and in methanol, soluble in ethanol (96 per cent).

IDENTIFICATION

First identification: A, D.

Second identification: B, C, D.

A. Infrared absorption spectrophotometry (2.2.24).

Preparation Discs.

Comparison *cloxacillin sodium CRS*.

B. Thin-layer chromatography (2.2.27).

Test solution Dissolve 25 mg of the substance to be examined in 5 mL of *water R*.

Reference solution (a) Dissolve 25 mg of *cloxacillin sodium CRS* in 5 mL of *water R*.

Reference solution (b) Dissolve 25 mg of *cloxacillin sodium CRS*, 25 mg of *dicloxacillin sodium CRS* and 25 mg of *flucloxacillin sodium CRS* in 5 mL of *water R*.

Plate *TLC silanised silica gel plate R*.

Mobile phase Mix 30 volumes of *acetone R* and 70 volumes of a 154 g/L solution of *ammonium acetate R*, then adjust to pH 5.0 with *glacial acetic acid R*.

Application 1 µL.

Development Over a path of 15 cm.

Drying In air.

Detection Expose to iodine vapour until the spots appear; examine in daylight.

System suitability Reference solution (b):

— the chromatogram shows 3 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Place about 2 mg in a test-tube about 150 mm long and 15 mm in diameter. Moisten with 0.05 mL of *water R* and add 2 mL of *sulfuric acid-formaldehyde reagent R*. Mix the contents of

the tube by swirling; the solution is slightly greenish-yellow. Place the test-tube in a water-bath for 1 min; the solution becomes yellow.
D. It gives reaction (a) of sodium (2.3.1).

TESTS

Solution S

Dissolve 2.50 g in *carbon dioxide-free water R* and dilute to 25.0 mL with the same solvent.

Appearance of solution

Solution S is clear (2.2.1) and its absorbance (2.2.25) at 430 nm is not greater than 0.04.

pH (2.2.3)

5.0 to 7.0 for solution S.

Specific optical rotation (2.2.7)

+ 160 to + 169 (anhydrous substance).

Dissolve 0.250 g in *water R* and dilute to 25.0 mL with the same solvent.

Related substances

Liquid chromatography (2.2.29).

Test solution (a) Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase.

Test solution (b) Dilute 5.0 mL of test solution (a) to 50.0 mL with the mobile phase.

Reference solution (a) Dissolve 50.0 mg of *cloxacillin sodium CRS* in the mobile phase and dilute to 50.0 mL with the mobile phase. Dilute 5.0 mL of this solution to 50.0 mL with the mobile phase.

Reference solution (b) Dilute 5.0 mL of test solution (b) to 50.0 mL with the mobile phase.

Reference solution (c) Dissolve 5 mg of *flucloxacillin sodium CRS* and 5 mg of *cloxacillin sodium CRS* in the mobile phase and dilute to 50.0 mL with the mobile phase.

Column:

— *size*: *l* = 0.25 m, Ø = 4 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase Mix 25 volumes of *acetonitrile R* and 75 volumes of a 2.7 g/L solution of *potassium dihydrogen phosphate R* adjusted to pH 5.0 with *dilute sodium hydroxide solution R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 225 nm.

Injection 20 µL of test solution (a) and reference solutions (b) and (c).

Run time 5 times the retention time of *cloxacillin*.

System suitability Reference solution (c):

— *resolution*: minimum 2.5 between the peaks due to *cloxacillin* (1st peak) and flucloxacillin (2nd peak).

Limits:

— *any impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

— *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (5.0 per cent);

— *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

_N,N_-Dimethylaniline (*2.4.26, Method B*)

Maximum 20 ppm.

2-Ethylhexanoic acid (*2.4.28*)

Maximum 0.8 per cent *m/m*.

Water (*2.5.12*)

3.0 per cent to 4.5 per cent, determined on 0.300 g.

Bacterial endotoxins (*2.6.14*)

Less than 0.20 IU/mg, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Injection Test solution (b) and reference solution (a).

System suitability:

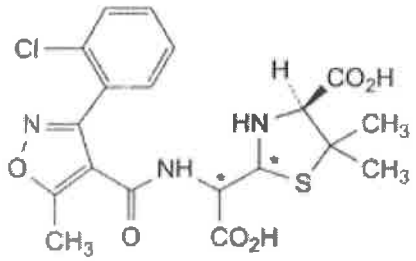
— *repeatability*: maximum relative standard deviation of 1.0 per cent after 6 injections of reference solution (a).

Calculate the percentage content of $C_{19}H_{17}ClN_3NaO_5S$ from the declared content of *cloxacillin sodium CRS*.

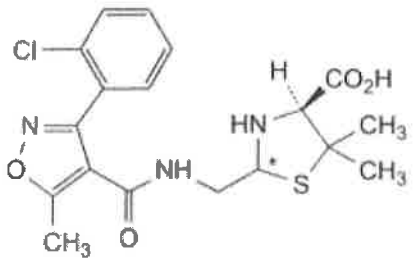
STORAGE

In an airtight container, at a temperature not exceeding 25 °C. If the substance is sterile, store in a sterile, airtight, tamper-evident container.

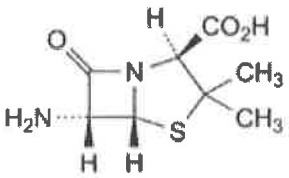
IMPURITIES



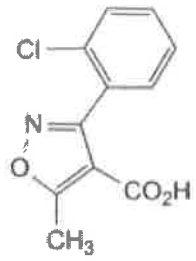
A. (4*S*)-2-[carboxy[[[3-(2-chlorophenyl)-5-methyl-1,2-oxazol-4-yl]carbonyl]amino]methyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid (penicilloic acid of *cloxacillin*),



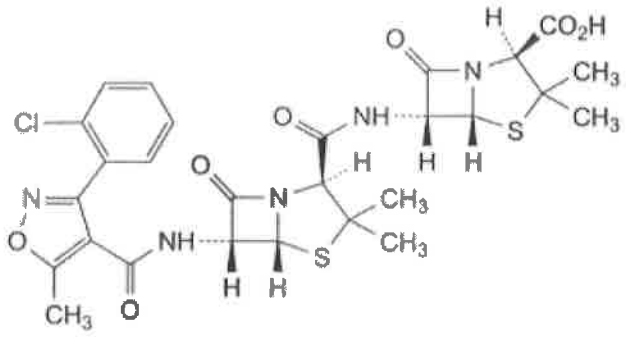
B. (2*RS*,4*S*)-2-[[[[3-(2-chlorophenyl)-5-methyl-1,2-oxazol-4-yl]carbonyl]amino]methyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid (penilloic acid of *cloxacillin*),



C. (2*S*,5*R*,6*R*)-6-amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-aminopenicillanic acid),



D. 3-(2-chlorophenyl)-5-methyl-1,2-oxazole-4-carboxylic acid,



E. (2*S*,5*R*,6*R*)-6-[[[(2*S*,5*R*,6*R*)-6-[[[3-(2-chlorophenyl)-5-methyl-1,2-oxazol-4-yl]carbonyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-yl]carbonyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-APA *cloxacillin* amide).

Ph Eur
