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

ENQUIRY REF. No.	KAPL/QAD/020/1384
DATE	23/09/2024
DUE DATE	26/09/2024 (13.00HRS)

Dear Sir,

Please submit your lowest and competitive offer in a SEALED ENVELOPE, DULY SUPERSCRIBING OUR ABOVE ENQUIRY REF. NO., DATE and DUE DATE on it/ OR MAIL, with other details of F.O.R terms, Taxes, Credit period, Delivery offered, Name of the Make, Detailed Specification etc., for below mentioned material/s

SL. NO.	ITEM CODE	ITEM DESCRIPTION	UOM	QTY
01	QSPHPL256	25CMX4.0MM ODS 5 MICRON C18 (MERCK)	NOS	02
02	QSPHPL411	15CMX4.6MM, 5MIC,PHENYL GROUP BONDED TO POROUS	NOS	01
03	QSPHPL212	YMC COLUMN C18 150X4.6 3UM ,PART NO-AS12S03-1546W	NOS	01

1)Please ensure that your offer reaches us on or before Due Date by courier OR speed post Or you can also mail us to our email: purenp@kaplindia.com
2) Please send your quotation mentioning item code

OTHER TERMS:

1. F.O.R TERMS : DOOR DELIVERY
2. GST % : PLEASE SPECIFY
3. PACKING & FORWARDING CHARGES : NOT APPLICABLE
4. CREDIT PERIOD : 30 DAYS
5. DELIVERY OFFERED :

NOTE: IN CASE YOU ARE NOT QUOTING PLEASE SEND THE REGRET LETTER.

Thanking you,

Yours faithfully,
For KARNATAKA ANTIBIOTICS
& PHARMACEUTICALS LIMITED

23/9

YUVARAJA M
DEPUTY MANAGER PURCHASE DEPT
MOB:9945317873

Benzylpenicillin Sodium BP

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 practically colourless. Place the test-tube on a water-bath for 1 min; a reddish-brown colour develops.

D. It gives reaction (a) of sodium (*2.3.1*).

TESTS

pH (*2.2.3*)

5.5 to 7.5.

Dissolve 2.0 g in *carbon dioxide-free water R* and dilute to 20 mL with the same solvent.

Appearance of solution

The solution is clear (*2.2.1*).

Dissolve 3.0 g in *water R* and dilute to 10 mL with the same solvent.

Related substances

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution (a) Dissolve 50.0 mg of the substance to be examined in *water R* and dilute to 50.0 mL with the same solvent.

Test solution (b) Dissolve 80.0 mg of the substance to be examined in *water R* and dilute to 20.0 mL with the same solvent.

Reference solution (a) Dissolve 50.0 mg of *benzylpenicillin sodium CRS* in *water R* and dilute to 50.0 mL with the same solvent.

Reference solution (b) Dissolve 5 mg of *benzylpenicillin for system suitability CRS* (containing impurities A, B, C, D, E, F, G and H) in 0.35 mL of *methanol R1* and add 0.65 mL of *water R*.

Reference solution (c) Dilute 1.0 mL of test solution (b) to 100.0 mL with *water R*.

Reference solution (d) Dilute 1.0 mL of reference solution (c) to 20.0 mL with *water R*.

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3 µm);

— *temperature*: 50 °C.

Mobile phase:

procaine benzylpenicillin BP

— *size*: *l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3 µm);

— *temperature*: 50 °C.

Mobile phase:

— *mobile phase A*: mix 10 volumes of a 68 g/L solution of *potassium dihydrogen phosphate R* adjusted to pH 3.4 with a 500 g/L solution of *phosphoric acid R*, 30 volumes of *methanol R1* and 60 volumes of *water for chromatography R*;

— *mobile phase B*: mix 10 volumes of a 68 g/L solution of *potassium dihydrogen phosphate R* adjusted to pH 3.4 with a 500 g/L solution of *phosphoric acid R*, 35 volumes of *water for chromatography R* and 55 volumes of *methanol R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 7	70	30
7 - 17	70 → 0	30 → 100
17 - 22	0	100

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 225 nm.

Injection 20 µL of test solution (b) and reference solutions (b), (d), (e) and (f).

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peak due to impurity A; use the chromatogram supplied with *procaine benzylpenicillin for peak identification A CRS* and the chromatogram obtained with reference solution (d) to identify the peaks due to impurities B, C, D, E, F, G, H, I and J.

Relative retention With reference to *benzylpenicillin* (retention time = about 7 min): procaine = about 0.19; impurity A = about 0.22; impurity D = about 0.33; impurity F = about 0.35; impurity B = about 0.48 and 0.55; impurity E = about 0.62; impurity C = about 0.81 and 0.83; impurity I = about 0.93; impurity G = about 1.47; impurity H = about 1.90; impurity J = about 2.37.

System suitability:

— *resolution*: minimum 1.0 between the peaks due to the epimers of impurity C and minimum 1.2 between the peaks due to impurities D and F in the chromatogram obtained with reference solution (d);

— *signal-to-noise ratio*: minimum 10 for the peak due to *benzylpenicillin* in the chromatogram obtained with reference solution (f).

Calculation of percentage contents:

— *correction factor*: multiply the peak area of impurity D by 0.4;

— for impurity A, use the concentration of impurity A in reference solution (b);

Standard stock solution 3: 1.0 mg/mL of USP Piperacillin RS in acetonitrile and *Diluent* (1:24). Dissolve first in acetonitrile, using about 4% of the final volume, and dilute with *Diluent* to volume.

System suitability solution: 6 µg/mL of tazobactam related compound A from *Standard stock solution 1* and 25 µg/mL of tazobactam from *Standard stock solution 2* in *Diluent*

Standard solution: 25 µg/mL of tazobactam from *Standard stock solution 2* and 0.2 mg/mL of piperacillin from *Standard stock solution 3* in *Mobile phase*. Refrigerate the solution immediately after preparation and during analysis, using a refrigerated autosampler set at 5 ± 3°. Analyze within 24 h of preparation.

Sample solution: Nominally 25 µg/mL of tazobactam and 0.2 mg/mL of piperacillin from Piperacillin and Tazobactam for Injection in *Mobile phase*. Refrigerate the solution immediately after preparation and during analysis, using a refrigerated autosampler set at 5 ± 3°. Analyze within 24 h of preparation.

Chromatographic system

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

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 15-cm; 3-µm packing L11

Flow rate: 1 mL/min

Injection volume: 20 µL

Autosampler temperature: 5 ± 3°

System suitability

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

Suitability requirements

Resolution: NLT 3 between tazobactam related compound A and tazobactam, *System suitability solution*

Tailing factor: NMT 1.8 for tazobactam and piperacillin, *Standard solution*

Relative standard deviation: NMT 2% for tazobactam and piperacillin, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Piperacillin and Tazobactam for Injection taken:

Printed on: Sat Jul 27 2024, 09:21:21 am
Printed by: Shashidhar B
Status: Currently Official on 27-Jul-2024
Official Date: Official as of 01-Mar-2018
Document Type: USP
DocId: GUID-155753B5-B047-4C1F-9305-2647222DE4FD_4_en-US
DOI: https://doi.org/10.31003/USPNF_M65215_04_01
DOI Ref: 3izf8

Piperacillin and Tazobactam for Injection

DEFINITION

Piperacillin and Tazobactam for Injection contains amounts of Piperacillin Sodium and Tazobactam Sodium equivalent to NLT 90.0% and NMT 110.0% of the labeled amounts of piperacillin ($C_{23}H_{27}N_5O_7S$) and tazobactam ($C_{10}H_{12}N_4O_5S$), the labeled amounts representing proportions of piperacillin to tazobactam of 8:1. It may contain small amounts of a suitable buffer and stabilizer.

IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

Buffer: 27.6 g/L of monobasic sodium phosphate

Solution A: 80 mL of 40% aqueous tetrabutylammonium hydroxide diluted with water to 100 mL

Mobile phase: Methanol, water, *Buffer*, and *Solution A* (510:432:50:8). Adjust with phosphoric acid to a pH of 5.5.

Standard solution: 0.1 mg/mL of USP Tazobactam RS and 1 mg/mL of USP Piperacillin RS in *Mobile phase*. Refrigerate the *Standard solution* immediately after preparation and during analysis, using a refrigerated autosampler set at 5 ± 3°. Analyze within 24 h of preparation.

Sample solution: Nominally 0.125 mg/mL of tazobactam and 1 mg/mL of piperacillin from Piperacillin and Tazobactam for Injection in *Mobile phase*. Refrigerate the *Sample solution* immediately after preparation and during analysis, using a refrigerated autosampler set at 5 ± 3°. Analyze within 24 h of preparation.

Chromatographic system

(See Chromatography ⟨621⟩, System Suitability.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

Autosampler temperature: 5 ± 3°

System suitability

[NOTE—The relative retention times for tazobactam and piperacillin are 0.36 and 1.0, respectively.]

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0 for tazobactam and piperacillin

Relative standard deviation: NMT 2.0% for tazobactam and piperacillin

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of piperacillin ($C_{23}H_{27}N_5O_7S$) in the portion of Piperacillin and Tazobactam for Injection taken:

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

r_U = peak response of piperacillin from the *Sample solution*

r_S = peak response of piperacillin from the *Standard solution*

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

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

P = potency of piperacillin in USP Piperacillin RS (µg/mg)

Piperacillin and Tazobactam for Injection

DEFINITION

Piperacillin and Tazobactam for Injection contains amounts of Piperacillin Sodium and Tazobactam Sodium equivalent to NLT 90.0% and NMT 110.0% of the labeled amounts of piperacillin ($C_{23}H_{27}N_5O_7S$) and tazobactam ($C_{10}H_{12}N_4O_5S$), the labeled amounts representing proportions of piperacillin to tazobactam of 8:1. It may contain small amounts of a suitable buffer and stabilizer.

IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

Buffer: 27.6 g/L of monobasic sodium phosphate

Solution A: 80 mL of 40% aqueous tetrabutylammonium hydroxide diluted with water to 100 mL

Mobile phase: Methanol, water, *Buffer*, and *Solution A* (510:432:50:8). Adjust with phosphoric acid to a pH of 5.5.

Standard solution: 0.1 mg/mL of USP Tazobactam RS and 1 mg/mL of USP Piperacillin RS in *Mobile phase*. Refrigerate the *Standard solution* immediately after preparation and during analysis, using a refrigerated autosampler set at 5 ± 3°. Analyze within 24 h of preparation.

Sample solution: Nominally 0.125 mg/mL of tazobactam and 1 mg/mL of piperacillin from Piperacillin and Tazobactam for Injection in *Mobile phase*. Refrigerate the *Sample solution* immediately after preparation and during analysis, using a refrigerated autosampler set at 5 ± 3°. Analyze within 24 h of preparation.

Chromatographic system

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (F_1/F_2) \times 100$$

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

r_S = peak response of piperacillin from the *Standard solution*

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

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

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

F_1 = correction factor, 0.001 mg/µg

F_2 = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor[a]	Acceptance Criteria, NMT (%)[a]
Tazobactam related compound A[b]	0.12	0.75	1.0
Tazobactam	0.25	—	—
Piperacillin impurity 4[c]	0.31	1.0	1.0

Name	Relative Retention Time	Relative Response Factor[a]	Acceptance Criteria, NMT (%)[a]
Piperacillin penilloic acid[d,e]	0.36	1.0	1.0
Piperacillin penicilloic acid[d,f]	0.51	0.56	5.0
Acetylated penicilloic acid of piperacillin[g]	0.55	1.0	1.0
Piperacillin impurity 5[c]	0.62	1.0	1.0
Piperacillin impurity 6[c]	0.67	1.0	1.0
Piperacillin	1.0	—	—
Any individual unspecified impurity	—	1.0	1.0
Total impurities[h]	—	—	5.0

[a] Calculated relative to the peak area of piperacillin.

[b] (2*S*,3*S*)-2-Amino-3-methyl-3-sulfino-4-(1*H*-1,2,3-triazol-1-yl)butyric acid.

[c] Specified unidentified impurities.

[d] This compound has two epimers that usually co-elute but that may be separated as a result of minor changes in the chromatographic conditions.

[e] (4*S*)-2-{[2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[f] (2*R*,4*S*)-2-{(1*R*)-Carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[g] (2*R*,4*S*)-3-Acetyl-2-{(1*R*)-carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[h] Total impurities does not include piperacillin penicilloic acid.

• **Organic Impurities, Procedure 2**

Organic Impurities, Procedure 2 is recommended when the impurity profile includes piperacillin dimer ethyl ester and piperacillin dimer thiazolamide derivative.

Solution A: 3.12 g/L of monobasic sodium phosphate. Adjust with phosphoric acid to a pH of 3.5.

Solution B: Methanol

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	90	10
5	85	15
10	65	35
35	55	45
60	35	65
65	90	10
75	90	10

System suitability solution 1: 10 µg/mL of USP Amoxicillin Related Compound A RS and 6 µg/mL of USP Tazobactam Related Compound A RS in *Solution A*. Refrigerate *System suitability solution 1* immediately after preparation and during analysis, using a refrigerated autosampler set at 5 ± 3°. Analyze within 24 h of preparation.

System suitability solution 2: 0.2 mg/mL each of USP Piperacillin RS and USP Tazobactam RS in a mixture of methanol and *Solution A* (30:70). Prepare the solution by dissolving the compounds in methanol and diluting with *Solution A* to volume. Refrigerate *System suitability solution 2* immediately after preparation and during analysis, using a refrigerated autosampler set at 5 ± 3°. Analyze within 24 h of preparation.

Sample solution: Nominally 2 mg/mL of piperacillin and 0.25 mg/mL of tazobactam from Piperacillin and Tazobactam for Injection in *Solution A*. Refrigerate the *Sample solution* immediately after preparation and during analysis, using a refrigerated autosampler set at 5 ± 3°. Analyze within 24 h of preparation.

Chromatographic system

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

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Temperatures

Column: 30°

Autosampler: 5 ± 3°

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution 1* and *System suitability solution 2*

Suitability requirements

Resolution: NLT 1.5 between tazobactam related compound A and amoxicillin related compound A, *System suitability solution 1*

Tailing factor: NMT 2.0 for the piperacillin and tazobactam peaks, *System suitability solution 2*

Relative standard deviation: NMT 10.0% for the piperacillin and tazobactam peaks, *System suitability solution 2*

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Piperacillin and Tazobactam for Injection taken:

$$\text{Result} = (r_U/r_T) \times 100$$

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

r_T = sum of the responses of all peaks from the *Sample solution*

Acceptance criteria: See *Table 3*.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tazobactam related compound A[a]	0.11	0.3
Amoxicillin related compound A[b]	0.13	0.2
Piperacillin related compound E[c]	0.17	0.8
Tazobactam	0.25	—
Formyl penicillamine[d]	0.34	0.2
Ampicillin	0.45	0.2
Piperazinedionecarbonyl D-phenylglycine[e]	0.53	0.2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Acetylated penicilloic acid of piperacillin[f]	0.64	0.5
Piperacillin penicilloic acid, isomer 1[g]	0.74	0.15
Piperacillin penicilloic acid, isomer 2[h]	0.78	1.5
L- Piperacillin[i,j]	0.81	—
Piperacillin penilloic acid[k]	0.91	0.5
Piperacillin	1.0	—
Piperacillin methyl ester[j,l]	1.2	—
Piperacillin dimer ethyl ester[m]	1.3	0.2
Piperacillin dimer thiazolamide derivative[n]	1.5	0.2
Piperacillin penicillamide[o]	1.6	0.3
Piperacillin dimer[p]	1.7	0.4

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Piperacillin ylampicillin[g]	1.9	0.3
Any individual unspecified impurity	—	0.1
Total impurities	—	4.0

[a] (2*S*,3*S*)-2-Amino-3-methyl-3-sulfino-4-(1*H*-1,2,3-triazol-1-yl)butyric acid.

[b] 6-Aminopenicillanic acid; (2*S*,5*R*,6*R*)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

[c] 1-Ethylpiperazine-2,3-dione.

[d] 2-Formamido-3-mercapto-3-methylbutanoic acid.

[e] (*R*)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetic acid.

[f] *N*-Acetyl piperacillin open ring; (2*R*,4*S*)-3-Acetyl-2-{(1*R*)-carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[g] (2*S*,4*S*)-2-{(1*R*)-Carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[h] Piperacillin open ring; (2*R*,4*S*)-2-{(1*R*)-Carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[i] (2*S*,5*R*,6*R*)-6-[(*S*)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

[j] Process impurities that are controlled in the drug substance are not to be reported. They are listed here for information only.

[k] Piperacillin penilloic analog; (4*S*)-2-{[2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[l] (2*S*,5*R*,6*R*)-Methyl 6-[(*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate.

[m] 2-(((3-Acetyl-4-(ethoxycarbonyl)-5,5-dimethylthiazolidin-2-yl)methyl)amino)-2-oxo-1-phenylethyl 6-(2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate.

[n] (2*R*,4*S*)-2-{(*R*)-Carboxy[(*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-3-((2*R*,4*S*)-2-{(*R*)-carboxy[(*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-5,5-dimethylthiazolidine-4-carbonyl)-5,5-dimethylthiazolidine-4-carboxylic acid.

[o] (2*S*,5*R*,6*R*)-6-{(2*S*,5*R*,6*R*)-6-[(*R*)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

[p] (2*R*,4*S*)-2-{(*R*)-Carboxy[(*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-3-{(2*S*,5*R*,6*R*)-6-[(*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carbonyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[q] Piperacillin amide dimer; (2*S*,5*R*,6*R*)-6-((*R*)-2-{(2*S*,5*R*,6*R*)-6-[(*R*)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido}-2-phenyl acetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carb oxylic acid.

- **Organic Impurities, Procedure 3**

Organic Impurities, Procedure 3 is recommended when the impurity profile includes piperacillin penicillenic acid and piperazinedionecarbonyl D-phenylglycylglycine.

Buffer: 27.6 g/L of monobasic sodium phosphate dihydrate

Solution A: 0.4 M aqueous tetrabutylammonium hydroxide

Solution B: Methanol, *Solution A*, *Buffer*, and water (275:3:100:622). Adjust with phosphoric acid to a pH of 5.5.

Solution C: Methanol, *Solution A*, *Buffer*, and water (615:3:100:282). Adjust with phosphoric acid to a pH of 5.5.

Mobile phase: See *Table 4*.

Table 4

Time (min)	Solution B (%)	Solution C (%)
0	100	0
6	100	0
55	71	29
73	10	90
85	10	90

System suitability solution: 60 µg/mL of USP Piperacillin Related Compound E RS, 0.1 mg/mL of USP Tazobactam Related Compound A RS, and 0.76 mg/mL of USP Tazobactam RS in *Solution C*

Standard solution 1: 6 mg/mL of USP Piperacillin RS in *Solution C*

Standard solution 2: 0.06 mg/mL of USP Piperacillin RS in *Solution C*

Sample solution: Nominally 5.1 mg/mL of piperacillin and 0.64 mg/mL of tazobactam from Piperacillin and Tazobactam for Injection in water. Refrigerate the *Sample solution* immediately after preparation and during analysis, using a refrigerated autosampler set at 4°. Analyze within 10 h of preparation.

Chromatographic system

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

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Temperatures

Column: 40°

Autosampler: 4°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution 2*

Suitability requirements

Resolution: NLT 1.5 between tazobactam related compound A and piperacillin related compound E, *System suitability solution*

Tailing factor: NMT 2.0 for piperacillin , *Standard solution 2*

Relative standard deviation: NMT 10.0% for the tazobactam peak, *System suitability solution*

Analysis

Samples: *Standard solution 2* and *Sample solution*

Calculate the percentage of each impurity in the portion of Piperacillin and Tazobactam for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (F_1/F_2) \times 100$$

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

r_S = peak response of piperacillin from *Standard solution 2*

C_S = concentration of USP Piperacillin RS in *Standard solution 2* (mg/mL)

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

P = potency of piperacillin in USP Piperacillin RS (µg/mg)

F_1 = correction factor, 0.001 mg/µg

F_2 = relative response factor (see *Table 5*)

Acceptance criteria: See *Table 5*. Disregard peaks that are 0.05 times the response of the peak in *Standard solution 2*. Disregard peaks that elute after piperacillin ylampicillin.

Table 5

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Piperacillin related compound E[a]	0.05	2.4	0.5
Tazobactam related compound A[b]	0.06	0.52	1.0
Tazobactam	0.09	—	—
Formyl penicillamine[c]	0.12	0.31	0.2
Ampicillin	0.14	0.79	0.3
Piperazinedionecarbonyl D-phenylglycine[d]	0.30	1.0	0.5
Piperazinedionecarbonyl D-phenylglycylglycine[e]	0.36	1.0	0.2
Acetylated penicilloic acids of piperacillin[f]	0.57	1.0	0.3
Piperacillin penicillenic acid[g]	0.60	1.0	0.2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Ampicillin hydantoin analog[h]	0.65	1.0	0.3
Piperacillin penicilloic acid, isomer 1[i]	0.71	1.0	
Piperacillin penicilloic acid, isomer 2[j,k]	0.83	1.0	3.0
Piperacillin oxalylamide[l]	0.75	1.0	0.2
L-Piperacillin[m,n]	0.80	1.0	—
	0.87	1.0	
Piperacillin penilloic acids[o,p]	0.92	1.0	1.0
Piperacillin	1.0	—	—
Piperazinedionecarbonyl D-phenyl glycylampicillin[n,q]	1.26	1.0	—
Open ring piperacillinylampicillin[n,r]	1.36	1.0	—

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Piperacillin penicillamide[s]	1.38	1.0	0.2
Piperacillin dimer[t]	1.41	1.0	0.5
Piperacillin ylampicillin[u]	1.54	1.0	1.0
Any individual unspecified impurity	–	1.0	0.1

[a] 1-Ethylpiperazine-2,3-dione.

[b] (2*S*,3*S*)-2-Amino-3-methyl-3-sulfino-4-(1*H*-1,2,3-triazol-1-yl)butyric acid.

[c] 2-Formamido-3-mercapto-3-methylbutanoic acid.

[d] (*R*)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetic acid.

[e] (*R*)-2-[2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenyl acetamido]acetic acid.

[f] (2*R*,4*S*)-3-Acetyl-2-{(1*R*)-carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[g] 2-{[(*E*)-{2-[(*R*)-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)(phenyl)methyl]-5-oxooxazol-4(5*H*)-ylidene}methyl]amino}-3-mercapto-3-methylbutanoic acid.

[h] (2*S*,5*R*,6*R*)-6-(2,5-Dioxo-4-phenylimidazolidin-1-yl)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

[i] (2*S*,4*S*)-2-{(1*R*)-Carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[j] (2*R*,4*S*)-2-{(1*R*)-Carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[k] The limit is for the sum of the two epimers of piperacillin open ring.

[l] (2*S*,5*R*,6*R*)-6-(2-{3-[2-(1-Carboxy-*N*-ethylformamido)ethyl]ureido}-2-phenylacetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

[m] (2*S*,5*R*,6*R*)-6-[(*S*)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

[n] Process impurities that are controlled in the drug substance are not to be reported. They are listed here for information only.

[o] (4*S*)-2-{[2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[p] The limit is for the sum of the two isomers of piperacillin penilloic analog.

[q] (2*S*,5*R*,6*R*)-6-{(*R*)-2-[(*R*)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-2-phenylacetamido}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

[r] (2*S*,5*R*,6*R*)-6-{(2*R*)-2-(2-[(4*S*)-4-Carboxy-5,5-dimethylthiazolidin-2-yl]-2-[(*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]acetamido)-2-phenylacetamido}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

[s] (2*S*,5*R*,6*R*)-6-{(2*S*,5*R*,6*R*)-6-[(*R*)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

[t] (2*R*,4*S*)-2-{(*R*)-Carboxy[(*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-3-{(2*S*,5*R*,6*R*)-6-[(*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carbonyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[u] (2*S*,5*R*,6*R*)-6-((*R*)-2-{(2*S*,5*R*,6*R*)-6-[(*R*)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido}-2-phenylacetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

- **Organic Impurities, Procedure 4**

Organic Impurities, Procedure 4 is recommended when the impurity profile includes piperacillin sulfoxide and piperacillin methyl penicilloate.

Buffer: 4 g/L of monobasic sodium phosphate dihydrate

Solution A: Acetonitrile and *Buffer* (2:98) adjusted with 1 M sodium hydroxide to a pH of 6.0 ± 0.05

Solution B: Acetonitrile

Mobile phase: See *Table 6*.

Table 6

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Temperatures

Column: 30°

Autosampler: 2°–8°

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

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

Suitability requirements

Resolution: NLT 1.5 between tazobactam related compound A and amoxicillin related compound A, *System suitability solution*

Tailing factor: NMT 2.0 for the piperacillin and tazobactam peaks, *Standard solution*

Relative standard deviation: NMT 5.0% for the piperacillin and tazobactam peaks, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity other than tazobactam related compound A in the portion of Piperacillin and Tazobactam for Injection taken:

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

r_U = peak response of each impurity other than tazobactam related compound A from the *Sample solution*

r_S = peak response of piperacillin from the *Standard solution*

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

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

P = potency of piperacillin in USP Piperacillin RS (µg/mg)

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	90	10
25	82	18
30	75	25
40	50	50
45	50	50
50	100	0
60	100	0

System suitability solution: 10 µg/mL of USP Amoxicillin Related Compound A RS and 6 µg/mL of USP Tazobactam Related Compound A RS in *Buffer*

Standard stock solution: 1 mg/mL of USP Piperacillin RS and 36 µg/mL of USP Tazobactam RS, prepared as follows. Dissolve suitable amounts of USP Piperacillin RS and USP Tazobactam RS in a small amount of acetonitrile. Dilute with *Buffer* to volume.

Standard solution: 50 µg/mL of piperacillin and 1.8 µg/mL of tazobactam from *Standard stock solution* in *Buffer*

Sample solution: Nominally 5 mg/mL of piperacillin and 0.625 mg/mL of tazobactam from Piperacillin and Tazobactam for Injection in *Buffer*. Store the *Sample solution* at 2°–8°, and use within 1 h.

Chromatographic system

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

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Temperatures

Column: 30°

Autosampler: 2°–8°

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

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

Suitability requirements

Resolution: NLT 1.5 between tazobactam related compound A and amoxicillin related compound A, *System suitability solution*

Tailing factor: NMT 2.0 for the piperacillin and tazobactam peaks, *Standard solution*

Relative standard deviation: NMT 5.0% for the piperacillin and tazobactam peaks, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity other than tazobactam related compound A in the portion of Piperacillin and Tazobactam for Injection taken:

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

r_U = peak response of each impurity other than tazobactam related compound A from the *Sample solution*

r_S = peak response of piperacillin from the *Standard solution*

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

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

P = potency of piperacillin in USP Piperacillin RS (µg/mg)

F = conversion factor, 0.001 mg/µg

Calculate the percentage of tazobactam related compound A in the portion of Piperacillin and Tazobactam for Injection taken:

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

r_U = peak response of tazobactam related compound A from the *Sample solution*

r_S = peak response of tazobactam from the *Standard solution*

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

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

P = potency of tazobactam in USP Tazobactam RS (mg/mg)

Acceptance criteria: See *Table 7*.

Table 7

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Tazobactam related compound A[a]	0.08	1.0
Amoxicillin related compound A[b]	0.15	0.2
Piperacillin related compound E[c]	0.18	0.8
Tazobactam	0.25	—

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Ampicillin	0.51	0.2
Acetylated penicilloic acid of piperacillin[d]	0.59	0.6
Piperazinedionecarbonyl D-phenylglycine[e]	0.63	0.1
Piperacillin penicilloic acid, isomer 1[f]	0.65	
Piperacillin penicilloic acid, isomer 2[g]	0.74	2.0
Ampicillin hydantoin analog, isomer 1[h]	0.78	0.2
Ampicillin hydantoin analog, isomer 2[h]	0.80	0.15
Piperacillin sulfoxide[i]	0.90	0.15

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Piperacillin penilloic analog, isomer 1[j]	0.94	0.5
Piperacillin penilloic analog, isomer 2[j]	0.95	
Piperacillin methyl penicilloate[k,l]	0.98	–
Piperacillin	1.0	–
Piperacillin dimer[m]	1.2	0.3
Piperacillin ylampicillin[l,n]	1.31	–
Any individual unspecified impurity	–	0.10

[a] (2*S*,3*S*)-2-Amino-3-methyl-3-sulfino-4-(1*H*-1,2,3-triazol-1-yl)butyric acid.

[b] 6-Aminopenicillanic acid; (2*S*,5*R*,6*R*)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

[c] 1-Ethylpiperazine-2,3-dione.

[d] (2*R*,4*S*)-3-Acetyl-2-{(1*R*)-carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[e] (*R*)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetic acid.

[f] (2*S*,4*S*)-2-{(1*R*)-Carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[g] (2*R*,4*S*)-2-{(1*R*)-Carboxy[2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[h] (2*S*,5*R*,6*R*)-6-(2,5-Dioxo-4-phenylimidazolidin-1-yl)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

[i] (2*S*,5*R*,6*R*)-6-[(*R*)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4-oxide.

[j] (4*S*)-2-{[2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[k] (2*R*,4*S*)-2-{(*R*)-1-[(*R*)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-2-methoxy-2-oxoethyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[l] Process impurities that are controlled in the drug substance are not to be reported. They are listed here for information only.

[m] (2*R*,4*S*)-2-{(*R*)-Carboxy[(*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-3-{(2*S*,5*R*,6*R*)-6-[(*R*)-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carbonyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[n] (2*S*,5*R*,6*R*)-6-((*R*)-2-{(2*S*,5*R*,6*R*)-6-[(*R*)-2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido}-2-phenylacetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

SPECIFIC TESTS

• **Bacterial Endotoxins Test ⟨85⟩:** It contains NMT 0.08 USP Endotoxin Units in a portion equivalent to 1 mg of a mixture of piperacillin and tazobactam (0.89 and 0.11 mg, respectively).

• **Sterility Tests ⟨71⟩:** Meets the requirements

• **Particulate Matter in Injections ⟨788⟩:** Meets the requirements

• **pH ⟨791⟩**

Sample solution: Nominally 40 mg/mL of piperacillin

Acceptance criteria: 5.0–7.0

• **Water Determination ⟨921⟩, *Method I*:** NMT 2.5%

• **Other Requirements:** It meets the requirements in *Injections and Implanted Drug Products ⟨1⟩*.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve as described in *Packaging and Storage Requirements ⟨659⟩, Injection Packaging, Packaging for constitution*. Store at controlled room temperature.

Dissolve 62.5 mg in a 4 g/L solution of *potassium hydrogen phthalate R* and dilute to 25.0 mL with the same solution.

Related substances

Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 31.0 mg of the substance to be examined in mobile phase A and dilute to 50.0 mL with mobile phase A.

Test solution (b) Dissolve 31.0 mg of the substance to be examined in mobile phase A and dilute to 10.0 mL with mobile phase A. *Prepare immediately before use.*

Reference solution (a) Dissolve 27.0 mg of *anhydrous ampicillin CRS* in mobile phase A and dilute to 50.0 mL with mobile phase A.

Reference solution (b) Dissolve 2 mg of *cefradine CRS* in mobile phase A and dilute to 50 mL with mobile phase A. To 5 mL of this solution add 5 mL of reference solution (a).

Reference solution (c) Dilute 1.0 mL of reference solution (a) to 20.0 mL with mobile phase A.

Reference solution (d) To 0.20 g of the substance to be examined add 1.0 mL of *water R*. Heat the solution at 60 °C for 1 h. Dilute 0.5 mL of this solution to 50.0 mL with mobile phase A.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

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

Mobile phase:

— *mobile phase A*: mix 0.5 mL of *dilute acetic acid R*, 50 mL of *0.2 M potassium dihydrogen phosphate R* and 50 mL of *acetonitrile R*, then dilute to 1000 mL with *water for chromatography R*;

— *mobile phase B*: mix 0.5 mL of *dilute acetic acid R*, 50 mL of *0.2 M potassium dihydrogen phosphate R* and 400 mL of *acetonitrile R*, then dilute to 1000 mL with *water for chromatography R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - t_R	85	15
t_R - (t_R + 30)	85 → 0	15 → 100
(t_R + 30) - (t_R + 45)	0	100
(t_R + 45) - (t_R + 60)	85	15

t_R = retention time of *ampicillin* determined with reference solution (c)

If the mobile phase composition has been adjusted to achieve the required resolution, the adjusted composition will apply at time zero in the gradient and in the assay.