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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)

F = conversion factor, 0.001 mg/µg

Calculate the percentage of the labeled amount of tazobactam ($C_{10}H_{12}N_4O_5S$) 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 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: 90.0%–110.0%

PERFORMANCE TESTS

• **Uniformity of Dosage Units ⟨905⟩:** Meets the requirements

IMPURITIES

• **Organic Impurities, Procedure 1**

Organic Impurities, Procedure 1 is recommended when the impurity profile includes piperacillin impurities 4, 5, and 6.

Buffer: Dilute the contents of one vial of tetrabutylammonium hydrogen sulfate ion pairing reagent with water to 1 L.

Solution A: Phosphoric acid and water (1:4)

Mobile phase: Acetonitrile and *Buffer* (25:75). Adjust with *Solution A* to a pH of 3.8.

Diluent: Acetonitrile and water (25:75)

Standard stock solution 1: 60 µg/mL of USP Tazobactam Related Compound A RS in *Diluent*

Standard stock solution 2: 0.5 mg/mL of USP Tazobactam RS in *Diluent*

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:

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

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
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
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[i,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 (%)
Piperacillinylampicillin[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 piperacillinpenicillenic 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 piperacillinylampicillin.

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
Piperacillinpenicillenic acid[g]	0.60	1.0	0.2
Ampicillin hydantoin analog[h]	0.65	1.0	0.3
Piperacillin penicilloic acid, isomer 1[i]	0.71	1.0	3.0
Piperacillin penicilloic acid, isomer 2[j,k]	0.83	1.0	
Piperacillin oxalylamide[l]	0.75	1.0	0.2
L-Piperacillin[m,n]	0.80	1.0	—
Piperacillin penilloic acids[o,p]	0.87	1.0	1.0
	0.92	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
Piperacillinylampicillin[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

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	—
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	2.0
Piperacillin penicilloic acid, isomer 2[g]	0.74	
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
Piperacillinylampicillin[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.

• **Labeling:** Label it to indicate its sodium content. If a test for *Organic Impurities* other than *Procedure 1* is used, then the labeling states with which *Organic Impurities* test the article complies.

• **USP Reference Standards** ⟨11⟩

USP Amoxicillin Related Compound A RS

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

$C_8H_{12}N_2O_3S$ 216.26

USP Piperacillin RS

USP Piperacillin Related Compound E RS

1-Ethylpiperazine-2,3-dione.

$C_6H_{10}N_2O_2$ 142.16

USP Tazobactam RS

USP Tazobactam Related Compound A RS

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

$C_7H_{12}N_4O_4S$ 248.26

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

Topic/Question	Contact	Expert Committee
PIPERACILLIN AND TAZOBACTAM FOR INJECTION	Documentary Standards Support	SM12020 Small Molecules 1
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	SM12020 Small Molecules 1

Chromatographic Database Information: Chromatographic Database

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