Table 1 *(Continued)*

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Piperacillin related compound A[c]	0.62	1.4	1.0
Piperacillin	1.0	—	—

[a] 1-Ethyl-2,3-piperazinedione.
[b] 2-(3-Acetyl-4-carboxy-5,5-dimethyl-2-thiazolidinyl)-*N*-[*N*-[(4-ethyl-2,3-dioxo-1-piperazinyl)-carbonyl]-2-phenylglycyl]glycine.
[c] 4-Carboxy-α-[2-(4-ethyl-2,3-dioxo-1-piperazinecarboxamido-2-phenylacetamido]-5,5-dimethyl-2-thiazolidinacetic acid.

- **PIPERACILLIN RELATED COMPOUND D**

Mobile phase: Methanol, water, 0.2 M monobasic sodium phosphate, and 0.4 M tetrabutylammonium hydroxide (615:282:100:3). Adjust with phosphoric acid to a pH of 5.50 ± 0.02.

Standard solution: 0.04 mg/mL of USP Piperacillin RS in *Mobile phase*. Dissolve in a few drops of methanol, and dilute with *Mobile phase* to volume. Use this solution within 1 h.

Sample solution: 0.4 mg/mL of Piperacillin in *Mobile phase*. Dissolve in a few drops of methanol, and dilute with *Mobile phase* to volume. Use this solution within 1 h.

Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1 mL/min
Injection size: 10 µL

System suitability
Sample: *Standard solution*
[NOTE—See *Table 2* for relative retention times.]
Suitability requirements
Relative standard deviation: NMT 2%

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of piperacillin related compound D in the portion of Piperacillin taken:

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

r_U = peak response of piperacillin related compound D 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 = concentration of the *Sample solution* (mg/mL)
P = potency of piperacillin in USP Piperacillin RS (µg/mg)
F_1 = relative response factor (see *Table 2*)
F_2 = conversion factor, 0.001 mg/µg

Acceptance criteria See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Piperacillin	1.0	—	—
Piperacillin related compound D[a]	2.55	1.47	2.0
Total impurities[b]	—	—	3.8

[a] 6-[2-[6-[2-(4-Ethyl-2,3-dioxo-1-piperazinecarboxamido)-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.
[b] Total impurities is the sum of all impurities reported in the tests for *Ampicillin and Piperacillin Related Compounds A, B, and C* and *Piperacillin Related Compound D*.

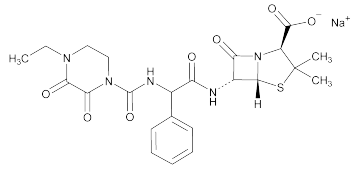
SPECIFIC TESTS

- **WATER DETERMINATION,** *Method I* ⟨**921**⟩**:** 2.0%–4.0%
- **OPTICAL ROTATION,** *Specific Rotation* ⟨**781S**⟩
  Sample solution: 40 mg/mL, in methanol
  Acceptance criteria: +155° to +175°
- **BACTERIAL ENDOTOXINS TEST** ⟨**85**⟩**:** Where the label states that Piperacillin is sterile or that it must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.07 USP Endotoxin Unit/mg of piperacillin.
- **STERILITY TESTS** ⟨**71**⟩**:** Where the label states that Piperacillin is sterile or that it must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements when tested as directed under *Test for Sterility of the Product to Be Examined, Membrane Filtration*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed 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 Endotoxin RS
  USP Piperacillin RS

Piperacillin Sodium



$C_{23}H_{26}N_5NaO_7S$ 539.54

4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 6-[[[[(4-ethyl-2,3-dioxo-1-piperazinyl)carbonyl]amino]phenylacetyl]amino]-3,3-dimethyl-7-oxo-, monosodium salt, [2*S*-[2α,5α,6β(*S**)]];
Sodium (2*S*,5*R*,6*R*)-6-[(*R*)-2-(4-ethyl-2,3-dioxo-1-piperazinecarboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate [59703-84-3].

DEFINITION

Piperacillin Sodium has a potency equivalent to NLT 863 µg/mg and NMT 1007 µg/mg of piperacillin ($C_{23}H_{27}N_5O_7S$), calculated on the anhydrous basis.

IDENTIFICATION

- **A.** The retention time of the major peak from the *Sample solution* corresponds to that from the *Standard solution*, and the chromatogram compares qualitatively to that from the *Standard solution* in the *Assay*.
- **B. IDENTIFICATION TESTS—GENERAL,** *Sodium* ⟨**191**⟩

ASSAY

- **PROCEDURE**

Mobile phase: Methanol, water, 0.2 M monobasic sodium phosphate, and 0.4 M tetrabutylammonium hydroxide(450:447:100:3). Adjust with phosphoric acid to a pH of 5.50 ± 0.02.

System suitability solution: 0.1 mg/mL of USP Ampicillin RS and 0.2 mg/mL of USP Piperacillin RS in *Mobile phase*

Standard solution 1: 0.4 mg/mL of USP Piperacillin RS in *Mobile phase*. Dissolve in a few drops of methanol, and dilute with *Mobile phase* to volume. Use this solution within 1 h.

Sample solution: 0.4 mg/mL of Piperacillin Sodium in *Mobile phase*

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1 mL/min
Injection size: 10 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—See *Table 1* for relative retention times.]
Suitability requirements
Resolution: NLT 16 between ampicillin and piperacillin, *System suitability solution*
Tailing factor: NMT 1.2 for the piperacillin peak, *System suitability solution*
Relative standard deviation: NMT 2% for the piperacillin peak, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the potency, in µg/mg, of piperacillin ($C_{23}H_{27}N_5O_7S$) in the portion of Piperacillin 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 Piperacillin RS in the *Standard solution* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)
P = potency of piperacillin in USP Piperacillin RS (µg/mg)

Acceptance criteria: 863–1007 µg/mg on the anhydrous basis

IMPURITIES

- **PIPERACILLIN RELATED COMPOUNDS A AND C**

Mobile phase, Standard solution 1, and **Sample solution:** Prepare as directed in the *Assay*.

System suitability solution: 0.1 mg/mL of USP Ampicillin RS and 0.2 mg/mL of USP Piperacillin RS in *Mobile phase*

Standard solution 2: 0.04 mg/mL of USP Piperacillin RS in *Mobile phase*. Dissolve in a few drops of methanol, and dilute with *Mobile phase* to volume. Use this solution within 1 h.

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 220 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1 mL/min
Injection size: 10 µL

System suitability
Samples: *Standard solution 1* and *System suitability solution*
[NOTE—See *Table 1* for relative retention times.]
Suitability requirements
Resolution: NLT 16 between ampicillin and piperacillin, *System suitability solution*
Tailing factor: NMT 1.2 for the piperacillin peak, *System suitability solution*
Relative standard deviation: NMT 2% for the piperacillin peak, *Standard solution 1*

Analysis
Samples: *Standard solution 2* and *Sample solution*
Calculate the percentages of piperacillin related compounds A and C in the portion of Piperacillin Sodium taken:

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

r_U = peak response of piperacillin related compound A or C from the *Sample solution*
r_S = peak response of piperacillin from the *Standard solution 2*
C_S = concentration of USP Piperacillin RS in the *Standard solution 2* (mg/mL)
C_U = concentration of the *Sample solution* (mg/mL)
P = potency of piperacillin in USP Piperacillin RS (µg/mg)
F_1 = relative response factor (see *Table 1*)
F_2 = conversion factor, 0.001 mg/µg

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Piperacillin related compound B	0.24	—	—
Ampicillin	0.31	—	—
Piperacillin related compound C	0.37	0.93	1.0
Piperacillin related compound A	0.62	1.4	3.5
Piperacillin	1.0	—	—

SPECIFIC TESTS

- **PH** ⟨**791**⟩
Sample solution: 400 mg/mL
Acceptance criteria: 5.5–7.5
- **WATER DETERMINATION,** *Method I* ⟨**921**⟩
Test preparation: Proceed as described for hygroscopic substances.
Acceptance criteria: NMT 1.0%
- **BACTERIAL ENDOTOXINS TEST** ⟨**85**⟩**:** Where the label states that Piperacillin Sodium is sterile or that it must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.07 USP Endotoxin Unit/mg of piperacillin.
- **STERILITY TESTS** ⟨**71**⟩**:** Where the label states that Piperacillin Sodium is sterile or that it must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements when tested as directed under *Test for Sterility of the Product to Be Examined, Membrane Filtration*.

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 Endotoxin RS
USP Piperacillin RS

Piperacillin for Injection

» Piperacillin for Injection contains an amount of piperacillin sodium equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of piperacillin ($C_{23}H_{27}N_5O_7S$).

Packaging and storage—Preserve in *Containers for Sterile Solids* as described under *Injections* ⟨1⟩.

USP Reference standards ⟨11⟩**—**
USP Ampicillin RS
USP Endotoxin RS
USP Piperacillin RS

Constituted solution—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* ⟨1⟩.

Bacterial endotoxins ⟨85⟩**—**It contains not more than 0.07 USP Endotoxin Unit per mg of piperacillin.

Sterility ⟨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 4.8 and 6.8, in a solution containing 200 mg of piperacillin per mL.

Water, *Method I* ⟨921⟩: not more than 0.9%.

Particulate matter ⟨788⟩: meets the requirements for small volume injections.

Related compounds—

Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay* under *Piperacillin*.

Standard piperacillin solution—Proceed as directed in the *Related compounds*, *Test 1* under *Piperacillin*.

Test solution 1 and *Test solution 2*—Use *Assay preparation 1* and *Assay preparation 2*, respectively, and proceed as directed under the *Assay*.

Procedure—Separately inject equal volumes (about 10 µL) of the *Test solutions* and the *Standard piperacillin solution*, and proceed as directed in the *Assay*. Calculate the percentage of piperacillin related compound A and piperacillin related compound C in the portion of Piperacillin for Injection taken by the formula:

$$0.1C(P / A)(RRF_i)(r_i / r_{Sp})$$

in which C is the concentration, in mg per mL, of USP Piperacillin RS in the *Standard piperacillin solution*, P is the designated potency, in µg of piperacillin per mg, of USP Piperacillin RS, A is the quantity, in mg, of piperacillin in each mL of *Test solution 1* or *Test solution 2*, RRF_i is the response factor of an individual piperacillin related compound relative to the response of piperacillin, specifically 1.4 for piperacillin related compound A and 0.93 for piperacillin related compound C, r_i is the response of each impurity peak, if any, observed in the chromatogram of the *Test solution* at a retention time corresponding to piperacillin related compound A or piperacillin related compound C, and r_{Sp} is the peak response of the piperacillin peak in the chromatogram of the *Standard piperacillin solution:* not more than 3.5% of piperacillin related compound A and not more than 1.0% of piperacillin related compound C is found.

Other requirements—It responds to the *Identification* test under *Piperacillin* and meets the requirements for *Uniformity of Dosage Units* ⟨905⟩ and *Labeling* under *Injections* ⟨1⟩.

Assay—

Mobile phase, Standard preparation, Resolution solution, and *Chromatographic system*—Proceed as directed in the *Assay* under *Piperacillin*.

Assay preparation 1 (where it is labeled for use as a single-dose container)—Constitute Piperacillin for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively with *Mobile phase* to obtain a solution containing about 0.4 mg of piperacillin per mL.

Assay preparation 2 (where the label states the quantity of piperacillin in a given volume of the constituted solution)—Constitute Piperacillin for Injection in 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 with *Mobile phase* to obtain a solution containing about 0.4 mg of piperacillin per mL.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparations* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg of piperacillin ($C_{23}H_{27}N_5O_7S$) in the container, or in the portion of constituted solution taken by the formula:

$$(L / D)(CP / 1000)(r_U / r_S)$$

in which L is the labeled quantity, in mg, of piperacillin in the container or in the volume of constituted solution taken, D is the concentration, in mg of piperacillin per mL, of *Assay preparation 1* or *Assay preparation 2*, based on the labeled quantity in the container or in the portion of constituted solution taken, respectively, and the extent of dilution, C is the concentration, in mg per mL, of USP Piperacillin RS in the *Standard preparation, P* is the designated potency, in µg of piperacillin per mg, of USP Piperacillin RS, and r_U and r_S are the piperacillin peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

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

• 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**

[NOTE—Refrigerate the *Standard solution* and the *Sample solution* immediately after preparation and during analysis, using a refrigerated autosampler set at 5 ± 3°. The solutions should be analyzed within 24 h of preparation.]

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

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

Mobile phase: Acetonitrile and *Solution B* (1:3), adjusted with *Solution A* to a pH of 3.8

Diluent: Acetonitrile and water (1:3)

Standard stock solution A: 0.06 mg/mL of USP Tazobactam Related Compound A RS in *Diluent*