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*

Standard stock solution B: 0.5 mg/mL of USP Tazobactam RS in *Diluent*
Standard stock solution C: 1.0 mg/mL of USP Piperacillin RS in acetonitrile and *Diluent* (1:24). [NOTE—Dissolve first in acetonitrile, using about 4% of the final volume, and dilute with *Diluent* to volume.]
System suitability solution: 0.006 mg/mL of tazobactam related compound A from *Standard stock solution A* and 0.025 mg/mL of tazobactam from *Standard stock solution B* in *Diluent*
Standard solution: 0.025 mg/mL of tazobactam from *Standard stock solution B* and 0.2 mg/mL of piperacillin from *Standard stock solution C* in *Mobile phase*
Sample solution: Equivalent to 0.025 mg/mL of tazobactam and 0.2 mg/mL of piperacillin from Piperacillin and Tazobactam for Injection in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 15-cm; 3-µm packing L11
Autosampler temperature: 5 ± 3°
Flow rate: 1 mL/min
Injection size: 20 µL
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 percentages of $C_{23}H_{27}N_5O_7S$ and $C_{10}H_{12}N_4O_5S$ 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 piperacillin or tazobactam from the *Sample solution*
r_S = peak response of piperacillin or tazobactam from the *Standard solution*
C_S = concentration of USP Piperacillin RS or USP Tazobactam RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of piperacillin or tazobactam in the *Sample solution* (mg/mL)
P = potency of piperacillin or tazobactam in USP Piperacillin RS or USP Tazobactam RS, respectively (mg/mg)
Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meets the requirements

IMPURITIES

Organic Impurities

- **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.]
Mobile phase, Diluent, System suitability solution, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each impurity in the portion of Piperacillin and Tazobactam for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/W_U) \times P \times D \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_S = response of piperacillin from the *Standard solution*
C_S = concentration of USP Piperacillin RS in the *Standard solution* (mg/mL)
W_U = weight of product used to prepare the *Sample solution* (mg)
P = potency of USP Piperacillin RS (mg/mg)
D = dilution factor of the *Sample solution*
F = relative response factor (see *Impurity Table 1*)
Acceptance criteria
Individual impurities: See *Impurity Table 1*.
Total impurities: NMT 5.0%. [NOTE—Total impurities does not include piperacillin related compound A.]

Impurity 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 impurity 1[d,e]	0.36	1.0	1.0
Piperacillin related compound A[e,f]	0.51	0.56	5.0
Piperacillin related compound C[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

[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] (4*S*)-2-{[2-(4-Ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.
[e] This compound has two epimers that usually co-elute but that may be separated as a result of minor changes in the chromatographic conditions.
[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.

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST ⟨85⟩:** It contains NMT 0.08 USP Endotoxin Unit 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⟩:** 5.0–7.0, in a solution containing the equivalent of 40 mg/mL of piperacillin
- **WATER DETERMINATION**, *Method I* **⟨921⟩:** NMT 2.5%
- **OTHER REQUIREMENTS:** It meets the requirements under *Injections* ⟨1⟩.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve as described in *Containers for Sterile Solids* under *Injections* ⟨1⟩, *Packaging*. Store at controlled room temperature.

- **LABELING:** Label it to indicate its sodium content.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Endotoxin RS
  USP Piperacillin RS
  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

Piperazine

$C_4H_{10}N_2$ 86.14
Piperazine.
Piperazine [110-85-0].

» Piperazine contains not less than 98.0 percent and not more than 101.0 percent of $C_4H_{10}N_2$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers, protected from light.

USP Reference standards ⟨11⟩**—**
USP Piperazine RS

Color of solution—Dissolve 10.0 g in water, and dilute with water to 50.0 mL: the solution has no more color than a standard solution prepared by adding 2.0 mL of ferric chloride CS to water and diluting with water to 50.0 mL, when compared in matched color-comparison tubes.

Identification—

A: *Infrared Absorption* ⟨197M⟩.

B: In the test for *Chromatographic purity,* the principal spot in the chromatogram of *Test solution 2,* observed after spraying with the ninhydrin solutions, corresponds in R_F value, color, and size to that in the chromatogram of *Standard solution 1*.

Melting range ⟨741⟩: between 109° and 113°.

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

Chromatographic purity—

Solvent—Prepare a mixture of 13.5 N ammonium hydroxide and dehydrated alcohol (3:2).

Standard solution 1—Prepare a solution of USP Piperazine RS in *Solvent* containing 10 mg per mL.

Standard solution 2—Prepare a solution of ethylenediamine in *Solvent* containing 0.25 mg per mL.

Standard solution 3—Prepare a solution of triethylenediamine in *Solvent* containing 0.25 mg per mL.

Resolution solution—Prepare a solution in *Solvent* containing 0.25 mg of triethylenediamine and 10 mg of USP Piperazine RS per mL.

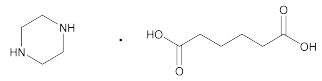
Test solution 1—Prepare a solution of Piperazine in *Solvent* containing 100 mg per mL.

Test solution 2—Mix 1 mL of *Test solution 1* and 9 mL of *Solvent*.

Procedure—Apply separate 5-µL portions of *Standard solution 1, Standard solution 2, Standard solution 3, Resolution solution, Test solution 1,* and *Test solution 2* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩), coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the chromatograms in a solvent system consisting of a freshly prepared mixture of acetone and 13.5 N ammonium hydroxide (80:20) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and dry the plate at 105°. Spray the plate with a 0.3% (w/v) solution of ninhydrin in a mixture of butyl alcohol and glacial acetic acid (100:3). Spray the plate again with a 0.15% (w/v) solution of ninhydrin in dehydrated alcohol, dry the plate at 105° for 10 minutes, and examine the plate: any secondary spot in the chromatogram obtained from *Test solution 1* is not more intense than the principal spot in the chromatogram obtained from *Standard solution 2* (0.25%). Spray the plate with 0.1 N iodine TS, allow to stand for 10 minutes, and examine the plate: any spot corresponding to triethylenediamine in the chromatogram obtained from *Test solution 1* is not more intense than the principal spot in the chromatogram obtained from *Standard solution 3* (0.25%). In a valid test, the chromatogram obtained from the *Resolution solution* shows a spot due to triethylenediamine clearly separated from the principal spot. Disregard any spot at the origin of any chromatogram.

Assay—Weigh accurately about 150 mg of Piperazine, and dissolve in 75 mL of glacial acetic acid. Titrate potentiometrically with 0.1 N perchloric acid VS, using a silver-glass electrode system. As the endpoint is approached, warm the solution to 60° to 70°, then complete the titration. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 4.307 mg of $C_4H_{10}N_2$.

Piperazine Adipate



$C_4H_{10}N_2 \cdot C_6H_{10}O_4$ 232.3
Piperazine, compound with 1,4-butanediacarboxylic acid (1:1).
Piperazine, compound with hexanedioic acid (1:1) [142-88-1].

» Piperazine Adipate contains not less than 98.0 percent and not more than 101.0 percent of $C_4H_{10}N_2 \cdot C_6H_{10}O_4$, calculated on the anhydrous basis.

Packaging and storage—Preserve in well-closed containers, and store at room temperature.

Labeling—Label it to indicate that it is for veterinary use only.

USP Reference standards ⟨11⟩**—**
USP Piperazine Adipate RS

Identification—

A: *Infrared Absorption* ⟨197K⟩.

B: In the test for *Chromatographic purity,* the principal spot in the chromatogram obtained from *Test solution 2* observed after spraying with the ninhydrin solutions corresponds in R_F value, color, and size to that in the chromatogram obtained from *Standard solution 1*.

C: To 10 mL of a 1 in 20 solution of it add 5 mL of hydrochloric acid, and extract with three 10-mL portions of ether. Evaporate the combined ether extracts to dryness, wash the residue with water, and dry at 105°: the residue of adipic acid so obtained melts at between 150° and 154°.

Water, *Method I* ⟨921⟩**:** not more than 0.5%.

Residue on ignition ⟨281⟩**:** not more than 0.1%.

Chromatographic purity—

Solvent—Prepare a mixture of 13.5 N ammonium hydroxide and dehydrated alcohol (3:2).

Test solution 1—Prepare a solution of Piperazine Adipate in *Solvent* containing 100 mg per mL.

Test solution 2—Mix 1 mL of *Test solution 1* and 9 mL of *Solvent*.

Standard solution 1—Prepare a solution of USP Piperazine Adipate RS in *Solvent* containing 10 mg per mL.