Spectinomycin Hydrochloride has a potency equivalent to not less than 603 μg of spectinomycin (C14H24N2O7) per mg.

Packaging and storage—Preserve in tight containers.

Labeling—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

**USP Reference standards** (11)—
USP Spectinomycin Hydrochloride RS
USP Endotoxin RS

**Identification, Infrared Absorption** (197M)—Do not dry specimen.

Crystallinity (695): meets the requirements.

Bacterial endotoxins (RS)—Where the label states that Spectinomycin Hydrochloride is sterile or that it must be subjected to further processing during the preparation of injectable dosage forms, it contains not more than 0.09 USP Endotoxin Unit per mg of spectinomycin.

Sterility (71)—Where the label states that Spectinomycin Hydrochloride is sterile, it meets the requirements when tested as directed for Membrane Filtration under Test for Sterility of the Product to be Examined.

**pH** (791): between 3.8 and 5.6, in a solution containing 10 mg per mL.

Water, Method I (921): between 16.0% and 20.0%

Residue on ignition (281): not more than 1.0%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid.

**Assay**—
Internal standard solution—Dissolve triphenylantimony in dimethylformamide to obtain a solution containing about 2 mg per mL.

Standard preparation—Transfer about 30 mg of USP Spectinomycin Hydrochloride RS, accurately weighed, to a glass-stoppered, 25-mL conical flask. Add 10.0 mL of Internal standard solution and 1.0 mL of hexamethyldisilazane, and shake intermittently for 1 hour.

Assay preparation—Proceed as directed under Standard preparation using Spectinomycin Hydrochloride.

Chromatographic system (see Chromatography (621))—The gas chromatograph is equipped with a flame-ionization detector and contains a 3-mm × 60-cm glass column packed with 5 percent phase G27 on 80- to 100-mesh support S1AB. The column and detector are maintained at about 190° and 220°, respectively, and the injection port at about 215°; and dry helium is used as the carrier gas at a flow rate of about 45 mL per minute. Chromatograph the Standard preparation, and record the chromatogram as directed for Procedure: the resolution, Rₚ between the major peaks is not less than 2.0; and the relative standard deviation of the peak response ratios, Rₛ, from replicate injections of the Standard preparation is not more than 3.5%.

Procedure—Separately inject equal volumes (about 1 μL) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the ratio, Rₛ/Rₚ, of the response of the spectinomycin peak to the response of the internal standard peak in the chromatogram from the Assay preparation, and similarly calculate the ratio, Rₛ, in the chromatogram from the Standard preparation. Calculate the quantity, in μg of C₁₄H₂₄N₂O₇ in the portion of Spectinomycin Hydrochloride taken to prepare the Assay preparation by the formula:

$$P(Wₛ/Rₛ/Rₚ)$$

in which $P$ is the potency of USP Spectinomycin Hydrochloride RS, in μg of spectinomycin per mg; and $Wₛ$ is the weight, in

<table>
<thead>
<tr>
<th>Carbon-Chain Length</th>
<th>Number of Double Bonds</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>3</td>
<td>5.0–11.0</td>
</tr>
<tr>
<td>20</td>
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<td>≤1.0</td>
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<tr>
<td>20</td>
<td>1</td>
<td>≤1.0</td>
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<tr>
<td>22</td>
<td>0</td>
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<tr>
<td>22</td>
<td>1</td>
<td>≤0.3</td>
</tr>
<tr>
<td>24</td>
<td>0</td>
<td>≤0.5</td>
</tr>
</tbody>
</table>

**Unsaponifiable matter** (401): not more than 1.5%.

**Peroxide value** (401)—
Potassium iodide solution—Mix 60 mL of glacial acetic acid with 40 mL of chloroform.

Procedure—Transfer about 10 g of Oil, accurately weighed, to a conical flask, add 30 mL of Mixed solvent, swirl to dissolve, add 0.5 mL of Potassium iodide solution, swirl the flask for 1 minute, accurately timed, add 30 mL of water, and titrate with 0.01 N sodium thiosulfate VS, with vigorous agitation, to a light yellow color. Add 0.5 mL of starch TS, and continue the titration until the blue color has disappeared. Perform a blank test, and make any necessary correction. Calculate the peroxide content, in mEq per kg, taken by the formula:

$$1000VN/W$$

in which $V$ is the volume, in mL, of sodium thiosulfate required and $N$ is its normality; and $W$ is the weight, in g, of Oil taken.

The limit is 10.0.

**Water, Method lc** (921): not more than 0.1%.

**Alkaline impurities**—Mix 10 mL of acetone and 0.3 mL of water, and add 0.05 mL of bromophenol blue TS. If necessary, neutralize the solution to a green color with 0.01 N hydrochloric acid or 0.01 N sodium hydroxide. Add 10 mL of Soybean Oil, shake, and allow to stand. Titrate with 0.01 N hydrochloric acid or 0.01 N sodium hydroxide, and make any necessary correction. Calculate the alkaline content, in mEq per kg, taken by the formula:

$$1000VN/W$$

The limit is 0.15.

**Peroxide value** (401)—

Potassium iodide solution—Mix 60 mL of glacial acetic acid with 40 mL of chloroform.

Procedure—Transfer about 10 g of Oil, accurately weighed, to a conical flask, add 30 mL of Mixed solvent, swirl to dissolve, add 0.5 mL of Potassium iodide solution, swirl the flask for 1 minute, accurately timed, add 30 mL of water, and titrate with 0.01 N sodium thiosulfate VS, with vigorous agitation, to a light yellow color. Add 0.5 mL of starch TS, and continue the titration until the blue color has disappeared. Perform a blank test, and make any necessary correction. Calculate the peroxide content, in mEq per kg, taken by the formula:

$$1000VN/W$$

in which $V$ is the volume, in mL, of sodium thiosulfate required and $N$ is its normality; and $W$ is the weight, in g, of Oil taken.

The limit is 10.0.

**Water, Method lc** (921): not more than 0.1%.

**Alkaline impurities**—Mix 10 mL of acetone and 0.3 mL of water, and add 0.05 mL of bromophenol blue TS. If necessary, neutralize the solution to a green color with 0.01 N hydrochloric acid or 0.01 N sodium hydroxide. Add 10 mL of Soybean Oil, shake, and allow to stand. Titrate with 0.01 N hydrochloric acid or 0.01 N sodium hydroxide, and make any necessary correction. Calculate the alkaline content, in mEq per kg, taken by the formula:

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Procedure—Transfer about 10 g of Oil, accurately weighed, to a conical flask, add 30 mL of Mixed solvent, swirl to dissolve, add 0.5 mL of Potassium iodide solution, swirl the flask for 1 minute, accurately timed, add 30 mL of water, and titrate with 0.01 N sodium thiosulfate VS, with vigorous agitation, to a light yellow color. Add 0.5 mL of starch TS, and continue the titration until the blue color has disappeared. Perform a blank test, and make any necessary correction. Calculate the peroxide content, in mEq per kg, taken by the formula:

$$1000VN/W$$

in which $V$ is the volume, in mL, of sodium thiosulfate required and $N$ is its normality; and $W$ is the weight, in g, of Oil taken.

The limit is 10.0.

**Water, Method lc** (921): not more than 0.1%.

**Alkaline impurities**—Mix 10 mL of acetone and 0.3 mL of water, and add 0.05 mL of bromophenol blue TS. If necessary, neutralize the solution to a green color with 0.01 N hydrochloric acid or 0.01 N sodium hydroxide. Add 10 mL of Soybean Oil, shake, and allow to stand. Titrate with 0.01 N hydrochloric acid or 0.01 N sodium hydroxide, and make any necessary correction. Calculate the alkaline content, in mEq per kg, taken by the formula:

$$1000VN/W$$

The limit is 0.15.

**Peroxide value** (401)—

Potassium iodide solution—Mix 60 mL of glacial acetic acid with 40 mL of chloroform.

Procedure—Transfer about 10 g of Oil, accurately weighed, to a conical flask, add 30 mL of Mixed solvent, swirl to dissolve, add 0.5 mL of Potassium iodide solution, swirl the flask for 1 minute, accurately timed, add 30 mL of water, and titrate with 0.01 N sodium thiosulfate VS, with vigorous agitation, to a light yellow color. Add 0.5 mL of starch TS, and continue the titration until the blue color has disappeared. Perform a blank test, and make any necessary correction. Calculate the peroxide content, in mEq per kg, taken by the formula:

$$1000VN/W$$

in which $V$ is the volume, in mL, of sodium thiosulfate required and $N$ is its normality; and $W$ is the weight, in g, of Oil taken.

The limit is 10.0.

**Water, Method lc** (921): not more than 0.1%.
mg, of USP Spectinomycin Hydrochloride RS taken from the Standard preparation; and the other terms are as defined above.

**Spectinomycin for Injectable Suspension**

» Spectinomycin for Injectable Suspension contains an amount of Spectinomycin Hydrochloride equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of spectinomycin (C₁₄H₂₄N₂O₇).

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

**USP Reference standards** (11)—
USP Endotoxin RS
USP Spectinomycin Hydrochloride RS

**Identification**—Infrared Absorption (197M). Do not dry specimen.

**pH** (791); between 4.0 and 7.0, in the suspension constituted as directed in the labeling.

**Other requirements**—It conforms to the Definition, and meets the requirements for Crystallinity, Bacterial endotoxins, Sterility, Water, and Residue on ignition under Spectinomycin Hydrochloride. It meets also the requirements for Uniformity of Dosage Units (905) and Labeling under Injections (1).

**Assay**—
Internal standard solution, Standard preparation, and Chromatographic system—Prepare as directed in the Assay under Spectinomycin Hydrochloride.

**Assay preparation 1**—Suspend the contents of 1 container of Spectinomycin for Injectable Suspension in water, and dilute quantitatively with water to obtain a stock solution containing about 20 mg of spectinomycin per mL. Transfer 1.0 mL of this solution to a glass-stoppered, 25-mL conical flask, and freeze-dry. Add 10.0 mL of Internal standard solution and 1.0 mL of hexamethyldisilazane, and shake intermittently for 1 hour.

**Assay preparation 2** (where the label states the quantity of spectinomycin in a given volume of constituted suspension)—Constitute 1 container of Spectinomycin for Injectable Suspension in a volume of water, accurately measured, corresponding to the volume of diluent specified in the labeling. Dilute an accurately measured portion of the constituted suspension quantitatively with water to obtain a stock solution containing about 20 mg of spectinomycin per mL. Transfer 1.0 mL of this solution to a glass-stoppered, 25-mL conical flask, and freeze-dry. Add 10.0 mL of Internal standard solution and 1.0 mL of hexamethyldisilazane, and shake intermittently for 1 hour.

**Procedure**—Proceed as directed in the Assay under Spectinomycin Hydrochloride. Calculate the quantity, in g, of C₁₄H₂₄N₂O₇ in the container of Spectinomycin for Injectable Suspension taken to prepare Assay preparation 1 taken by the formula:

\[
\frac{L_1}{D_1} \times \frac{P}{1000} \times \frac{W(W/r)}{R_1}
\]

in which \(L_1\) is the labeled quantity, in g, of C₁₄H₂₄N₂O₇ in the container, and \(D_1\) is the concentration, in mg per mL, of spectinomycin in the stock solution used to prepare Assay preparation 1, on the basis of the labeled quantity in the container and the extent of dilution, and the other terms are as defined therein. Calculate the quantity, in mg, of C₁₄H₂₄N₂O₇ in each mL of constituted Injectable Suspension taken to prepare Assay preparation 2 taken by the formula:

\[
\frac{L_2}{D_2} \times \frac{P}{1000} \times \frac{W(W/r)}{R_2}
\]

in which \(L_2\) is the labeled quantity, in mg, of C₁₄H₂₄N₂O₇ in each mL of constituted suspension of Spectinomycin for Inject-

able Suspension, and \(D_2\) is the concentration, in mg per mL, of spectinomycin in the stock solution used to prepare Assay preparation 2, on the basis of the labeled quantity in each mL of constituted suspension and the extent of dilution.

**Spectinomycin for Injectable Suspension**

C₂₄H₃₂O₄S

Pregn-4-ene-21-carboxylic acid, 7-(acetylthio)-17-hydroxy-3-oxo-α-lactone, (7α,17α)-
17-Hydroxy-7α-mercapto-3-oxo-17α-pregn-4-ene-21-carboxylic acid γ-lactone acetate [52-01-7].

**DEFINITION**
Spironolactone contains NLT 97.0% and NMT 103.0% of C₂₄H₃₂O₄S, calculated on the dried basis.

**IDENTIFICATION**

**Change to read:**

- **A. INFRARED ABSORPTION (197K)**
- **B. ULTRAVIOLET ABSORPTION (197U)**

**ASSAY**

**Procedure**

Mobile phase: Methanol and water (60:40)
Standard solution: 0.5 mg/mL of USP Spironolactone RS in a mixture of acetonitrile and water (1:1)
Sample solution: 0.5 mg/mL of USP Spironolactone RS in a mixture of acetonitrile and water (1:1)

**Chromatographic system**
(See Chromatography (621), System Suitability.)
Mode: LC
Detector: UV 230 nm
Column: 4.6-mm x 15-cm; packing L1
Flow rate: 1 mL/min
Injection size: 20 µL

**System suitability**

Sample: Standard solution
Suitability requirements:
- Tailing factor: NMT 2.0
- Relative standard deviation: NMT 1.5%

**Analysis**

Samples: Standard solution and Sample solution
Calculate the percentage of spironolactone (C₂₄H₃₂O₄S) in the portion of sample taken:

\[
\text{Result} = \left( \frac{r_2}{r_1} \right) \times \left( \frac{C_1}{C_2} \right) \times 100
\]

\(r_1\) = peak response from the Sample solution
\(r_2\) = peak response from the Standard solution

**Spironolactone**

C₂₄H₃₂O₄S

Pregn-4-ene-21-carboxylic acid, 7-(acetylthio)-17-hydroxy-3-oxo-γ-lactone, (7α,17α); 17-Hydroxy-7α-mercapto-3-oxo-17α-pregn-4-ene-21-carboxylic acid γ-lactone acetate [52-01-7].