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 ($C_{14}H_{24}N_2O_7$).

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 $C_{14}H_{24}N_2O_7$ in the container of Spectinomycin for Injectable Suspension taken to prepare *Assay preparation 1* taken by the formula:

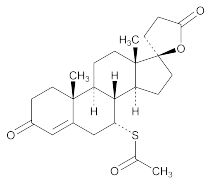
$$(L_1 / D_1)(P / 1000)(W_S)(R_U / R_S)$$

in which L_1 is the labeled quantity, in g, of $C_{14}H_{24}N_2O_7$ 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 $C_{14}H_{24}N_2O_7$ in each mL of constituted Injectable Suspension taken to prepare *Assay preparation 2* taken by the formula:

$$(L_2 / D_2)(P / 1000)(W_S)(R_U / R_S)$$

in which L_2 is the labeled quantity, in mg, of $C_{14}H_{24}N_2O_7$ in each mL of constituted suspension of Spectinomycin for Injectable 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.

Spironolactone



$C_{24}H_{32}O_4S$ 416.57
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].

DEFINITION
Spironolactone contains NLT 97.0% and NMT 103.0% of $C_{24}H_{32}O_4S$, calculated on the dried basis.

IDENTIFICATION

Change to read:

- **A. Infrared Absorption** ▲⟨197K⟩▲*USP35*
- **B. Ultraviolet Absorption** ⟨197U⟩
  Sample solution: 10 µg/mL in methanol
  Analytical wavelength: 238 nm
  Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.
- **C.**
  Sample solution: Add 100 mg to a mixture of 10 mL of water and 2 mL of 1 N sodium hydroxide.
  Analysis: Boil the mixture for 3 min, cool, and add 1 mL of glacial acetic acid and 1 mL of lead acetate TS.
  Acceptance criteria: A brown-to-black precipitate of lead sulfide is formed.

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 Spironolactone 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 × 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 ($C_{24}H_{32}O_4S$) in the portion of sample taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*

C_S = concentration of USP Spironolactone RS in the *Standard solution* (mg/mL)
C_U = concentration of Spironolactone in the *Sample solution* (mg/mL)
Acceptance criteria: 97.0%–103.0% on the dried basis

IMPURITIES

- **LIMIT OF MERCAPTO COMPOUNDS**
  Sample solution: Shake 2.0 g with 30 mL of water, and filter.
  Analysis: To 15 mL of the filtrate add 3 mL of starch TS, and titrate with 0.010 N iodine. Perform a blank determination, and make any necessary correction.
  Acceptance criteria: NMT 0.10 mL of 0.010 N iodine is consumed.
- **ORDINARY IMPURITIES** ⟨**466**⟩
  Standard solution: Chloroform
  Test solution: Chloroform
  Eluant: Butyl acetate
  Visualization: 5

SPECIFIC TESTS

Change to read:

- **OPTICAL ROTATION,** *Specific Rotation* ⟨**781S**⟩**:** ▲–41° to –45°▲*USP35*
  Sample solution: 10 mg/mL in ▲alcohol▲*USP35*
- **LOSS ON DRYING** ⟨**731**⟩**:** Dry a sample at 105° for 2 h: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Spironolactone RS

Spironolactone Tablets

» Spironolactone Tablets contain not less than 95.0 percent and not more than 105.0 percent of the labeled amount of spironolactone ($C_{24}H_{32}O_4S$).

Packaging and storage—Preserve in tight, light-resistant containers.

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

Identification—Mix a quantity of finely powdered Tablets, equivalent to about 100 mg of spironolactone, with 25 mL of methanol, and filter. Apply 10 µL of this solution and 10 µL of a solution of USP Spironolactone RS in methanol containing 4 mg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatogram in a solvent system consisting of a mixture of chloroform, ethyl acetate, and methanol (2:2:1) 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 allow the solvent to evaporate. Locate the spots on the plate by viewing under short-wavelength UV light: the R_F value of the principal spot obtained from the solution under test corresponds to that obtained from the Standard solution.

Dissolution ⟨711⟩**—**

Medium: 0.1 N hydrochloric acid containing 0.1% of sodium lauryl sulfate; 1000 mL.

Apparatus 2: 75 rpm.

Time: 60 minutes.

Procedure—Determine the amount of $C_{24}H_{32}O_4S$ dissolved using UV absorption at the wavelength of maximum absorbance at about 242 nm obtained on filtered portions of the solution under test, diluted with *Medium,* if necessary, in comparison with a Standard solution having a known concentration of USP Spironolactone RS in the same *Medium.* [NOTE—A volume of alcohol not exceeding 1% of the final volume of the solution may be used to prepare the Standard solution.]

Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{24}H_{32}O_4S$ is dissolved in 60 minutes.

Uniformity of dosage units ⟨905⟩**:** meet the requirements.

Assay—

Mobile phase, Standard preparation, and *Chromatographic system*—Prepare as directed in the *Assay* under *Spironolactone.*

Diluent—Prepare a mixture of acetonitrile and water (1:1).

Assay preparation—Accurately weigh not fewer than 10 Tablets, and transfer to a suitable volumetric flask. [NOTE—The target concentration is about 1 mg per mL.] Add a sufficient quantity of *Diluent,* shake for about 30 minutes, and sonicate for 30 minutes or until the Tablets are disintegrated. Cool the solution to room temperature, dilute with *Diluent* to volume, and centrifuge a suitable portion of the mixture. Quantitatively dilute a portion of this solution with *Diluent* to obtain a solution having a known concentration of about 0.5 mg of spironolactone per mL.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Spironolactone.* Calculate the quantity, in mg, of spironolactone ($C_{24}H_{32}O_4S$) in the portion of Tablets taken by the formula:

$$CD(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Spironolactone RS in the *Standard preparation; D* is the dilution factor for the *Assay preparation;* and r_U and r_S are the peak responses for spironolactone obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Spironolactone and Hydrochlorothiazide Tablets

» Spironolactone and Hydrochlorothiazide Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of spironolactone ($C_{24}H_{32}O_4S$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩**—**
USP Hydrochlorothiazide RS
USP Spironolactone RS

Identification—The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Dissolution ⟨711⟩**—**

Medium: 0.1 N hydrochloric acid containing 0.1% sodium lauryl sulfate; 900 mL.

Apparatus 2: 75 rpm.

Time: 60 minutes.

Determine the amounts of spironolactone and hydrochlorothiazide dissolved using the following method.

Standard solution—Prepare a solution of USP Spironolactone RS and USP Hydrochlorothiazide RS in a mixture of methanol and *Medium* (1:1) having accurately known concentrations of about 0.0125 mg of each per mL.