USP Reference standards ⟨11⟩—
USP Endotoxin RS

Identification—It responds to the *Identification* tests under *Dextrose* and under *Dehydrated Alcohol.*

Bacterial endotoxins ⟨85⟩**—**It contains not more than 0.5 USP Endotoxin Unit per mL.

pH ⟨791⟩**:** between 3.5 and 6.5, determined on a portion to which 0.30 mL of saturated potassium chloride solution has been added for each 100 mL and which previously has been diluted with water, if necessary, to a concentration of not more than 5% of dextrose.

Heavy metals ⟨231⟩**—**Transfer a volume of Injection, equivalent to 4.0 g of dextrose, to a vessel, and adjust the volume to 25 mL by evaporation or by addition of water, as necessary: the limit is 0.0005 *C*%, in which *C* is the labeled amount, in g, of $C_6H_{12}O_6 \cdot H_2O$ per mL of Injection.

Limit of 5-hydroxymethylfurfural and related substances—Dilute an accurately measured volume of Injection, equivalent to 1.0 g of $C_6H_{12}O_6 \cdot H_2O$, with water to 500.0 mL. Determine the absorbance of this solution in a 1-cm cell at 284 nm, with a suitable spectrophotometer, using water as the blank: the absorbance is not more than 0.25.

Other requirements—It meets the requirements under *Injections* ⟨1⟩.

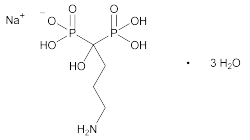
Assay for alcohol—Determine by *Method I—Distillation Method* under *Alcohol Determination* ⟨611⟩, using a 50.0-mL portion of Injection.

Assay for dextrose—Transfer an accurately measured volume of Injection, containing from 2 to 5 g of dextrose, to a 100-mL volumetric flask. Add 0.2 mL of 6 *N* ammonium hydroxide, dilute with water to volume, and mix. Determine the angular rotation in a suitable polarimeter tube (see *Optical Rotation* ⟨781⟩). Calculate the percentage (g per 100 mL) of dextrose ($C_6H_{12}O_6 \cdot H_2O$) in the portion of Injection taken by the formula:

$$(100/52.9)(198.17/180.16)AR$$

in which 100 is the percentage; 52.9 is the midpoint of the specific rotation range for anhydrous dextrose, in degrees; 198.17 and 180.16 are the molecular weights for dextrose monohydrate and anhydrous dextrose, respectively; *A* is 100 mm divided by the length of the polarimeter tube, in mm; and *R* is the observed rotation, in degrees.

Alendronate Sodium



$C_4H_{12}NNaO_7P_2 \cdot 3H_2O$ 325.12
Phosphonic acid, (4-amino-1-hydroxybutylidene)bis-, monosodium salt, trihydrate.
Sodium trihydrogen (4-amino-1-hydroxybutylidene) diphosphonate, trihydrate [121268-17-5].

» Alendronate Sodium contains not less than 98.0 percent and not more than 102.0 per cent of $C_4H_{12}NNaO_7P_2$, calculated on the dried basis.

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

USP Reference standards ⟨11⟩**—**
USP Alendronate Sodium RS

Identification—

A: *Infrared Absorption* ⟨197M⟩.

B: It meets the requirements of the flame test for *Sodium* ⟨191⟩.

Loss on drying ⟨731⟩**—**Dry it at a pressure not exceeding 5 mm of mercury at 140° to constant weight: it loses not less than 16.1% and not more than 17.1% of its weight.

Heavy metals, *Method III* ⟨231⟩**:** 0.001%.

Chromatographic purity—

Borate solution and *Diluent*—Prepare as directed in the *Assay*.

Buffer solution—Transfer 5.88 g of sodium citrate dihydrate and 2.84 g of anhydrous dibasic sodium phosphate to a 2-L volumetric flask, dilute with water to volume, and mix. Adjust with phosphoric acid to a pH of 8, and pass the solution through a filter having a 0.5-µm or finer porosity.

9-Fluorenylmethyl chloroformate solution—Prepare a solution in acetonitrile containing about 4 mg of 9-fluorenylmethyl chloroformate per mL. Prepare this solution fresh just prior to use.

Solution A—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (17:3).

Solution B—Prepare a filtered and degassed mixture of acetonitrile and *Buffer solution* (7:3).

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system.* Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard stock solution—Prepare a solution of USP Alendronate Sodium RS in *Diluent* having a known concentration of about 0.6 mg per mL.

Standard solution—Transfer 5.0 mL of the *Standard stock solution* to a 50-mL polypropylene, screw-cap centrifuge tube containing 5 mL of *Borate solution.* Add 5 mL of acetonitrile and 5 mL of *9-Fluorenylmethyl chloroformate solution,* and shake for 45 seconds. Allow to stand at room temperature for 30 minutes. Add 20 mL of methylene chloride, and shake vigorously for 1 minute. Centrifuge for 5 to 10 minutes, and use a portion of the clear upper aqueous layer.

Diluted standard solution—Dilute a portion of the *Standard stock solution* with *Diluent* to obtain a solution having a known concentration of about 0.6 µg per mL. Using 5 mL of this solution, proceed as directed for the *Standard solution,* beginning with "to a 50-mL polypropylene, screw-cap centrifuge tube."

Reagent blank—Using a 5.0-mL portion of *Diluent,* proceed as directed for *Standard solution,* beginning with "to a 50-mL polypropylene, screw-cap centrifuge tube."

Test solution—Transfer about 30 mg of Alendronate Sodium, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, and mix. Using a 5.0-mL volume of this solution, proceed as directed for *Standard solution,* beginning with "to a 50-mL polypropylene, screw-cap centrifuge tube."

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 266-nm detector and a 4.1-mm × 25-cm column that contains packing L21. The flow rate is about 1.8 mL per minute. The column temperature is maintained at about 45°. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0	100	0	equilibration
0–15	100→50	0→50	linear gradient
15–25	50→0	50→100	linear gradient
25–27	0→100	100→0	linear gradient
27–32	100	0	isocratic

Chromatograph the *Standard solution* and the *Diluted standard solution,* and record the peak responses as directed for *Procedure:* the tailing factor for the main peak in the chromatogram

of the *Standard solution* is not more than 2.0; and the peak at that locus in the chromatogram of the *Diluted standard solution* is detectable with a signal-to-noise ratio of not less than 3.

Procedure—Separately inject equal volumes (about 20 µL) of the *Test solution* and the *Reagent blank* into the chromatograph, record the chromatograms, and measure the responses for all the peaks. Disregard any peak corresponding to those obtained from the *Reagent blank*. Calculate the per centage of each impurity in the portion of Alendronate Sodium taken by the formula:

$$100(r_i / r_s)$$

in which r_i is the area of each impurity peak, and r_s is the sum of all impurity peaks and the main peak: not more than 0.1% of any individual impurity is found, and not more than 0.5% of total impurities is found.

Assay—

Buffer solution—Transfer 14.7 g of sodium citrate dihydrate and 7.05 g of anhydrous dibasic sodium phosphate to a 1-L volumetric flask, dilute with water to volume, mix, and adjust with phosphoric acid to a pH of 8.

Diluent—Dissolve 29.4 g of sodium citrate dihydrate in water in a 1-L volumetric flask, dilute with water to volume, and mix.

Borate solution—Dissolve 19.1 g of sodium borate in water in a 1-L volumetric flask, dilute with water to volume, and mix.

9-Fluorenylmethyl chloroformate solution—Prepare a solution in acetonitrile containing about 0.5 mg of 9-fluorenylmethyl chloroformate per mL. Prepare this solution fresh just prior to use.

Mobile phase—Prepare a filtered and degassed mixture of *Buffer solution,* acetonitrile, and methanol (70:25:5). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard stock preparation—Prepare a solution of USP Alendronate Sodium RS in *Diluent* having a known concentration of about 0.1 mg per mL. Calculate the concentration, C_S, of anhydrous alendronate sodium in this solution.

Standard preparation—Transfer 5.0 mL of the *Standard stock preparation* to a 50-mL polypropylene, screw-cap centrifuge tube containing 5 mL of *Borate solution.* Add 5 mL of *9-Fluorenylmethyl chloroformate solution,* and shake for 30 seconds. Allow to stand at room temperature for 25 minutes. Add 25 mL of methylene chloride, and shake vigorously for 1 minute. Centrifuge for 5 to 10 minutes. Use a portion of the clear upper aqueous layer.

Reagent blank—Using 5.0 mL of *Diluent,* proceed as directed for *Standard preparation,* beginning with "to a 50-mL polypropylene, screw-cap centrifuge tube."

Assay stock preparation—Transfer about 25 mg of Alendronate Sodium, accurately weighed, to a 250-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, and mix.

Assay preparation—Using 5.0 mL of the *Assay stock preparation,* proceed as directed for the *Standard preparation,* beginning with "to a 50-mL polypropylene, screw-cap centrifuge tube."

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 266-nm detector and a 4.1-mm × 25-cm column that contains packing L21. The flow rate is about 1.2 mL per minute. The column temperature is maintained at about 35 °. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the column efficiency is not less than 1500 theoretical plates, the tailing factor is not more than 1.5, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation,* the *Assay preparation,* and the *Reagent blank* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_4H_{12}NNaO_7P_2$ in the portion of Alendronate Sodium taken by the formula:

$$DC_S (r_U / r_S)$$

in which D is the dilution factor for the *Assay stock preparation;* C_S is as defined under the *Standard stock preparation;* and r_U and r_S are the peak area responses for alendronic acid obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Alendronate Sodium Tablets

DEFINITION

Alendronate Sodium Tablets contain an amount of Alendronate Sodium equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of alendronic acid ($C_4H_{13}NO_7P_2$).

IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

Solution A: 14.7 g/L of sodium citrate dihydrate and 7.05 g/L of anhydrous dibasic sodium phosphate in water. [NOTE—Adjust with phosphoric acid to a pH of 8.0 before bringing the solution to volume.]

Solution B: 38.1 g/L of sodium borate in water

Solution C: 1 mg/mL of 9-fluorenylmethyl chloroformate in acetonitrile. [NOTE—Prepare this solution fresh just before use.]

Mobile phase: Acetonitrile, methanol, and *Solution A* (20:5:75)

Diluent: 29.4 g/L of sodium citrate dihydrate in water

Standard stock solution: 0.03 mg/mL of anhydrous alendronate sodium in *Diluent*, from USP Alendronate Sodium RS

Standard solution: Transfer 5.0 mL of the *Standard stock solution* to a 50-mL polypropylene screw-cap centrifuge tube containing 5 mL of *Solution B*, and mix for 3 min. Add 4 mL of *Solution C*, and agitate for 30 s. Allow the solution to stand at room temperature for 25 min. Add 25 mL of methylene chloride, and agitate for 40 s. Centrifuge the mixture for 10 min. Use the clear upper aqueous layer.

Sample stock solution: Transfer NLT 10 Tablets to a 1000-mL volumetric flask. Add 500 mL of *Diluent*, shake by mechanical means for 30 min, and sonicate for 5 min. Dilute with *Diluent* to volume, and centrifuge a portion of this solution. Quantitatively dilute a portion of the clear supernatant to a concentration of 0.02–0.03 mg/mL of alendronic acid.

Sample solution: Transfer 5.0 mL of the *Sample stock solution* to a 50-mL polypropylene screw-cap centrifuge tube containing 5 mL of *Solution B*, and mix for 3 min. Add 4 mL of *Solution C*, and agitate for 30 s. Allow the solution to stand at room temperature for 25 min. Add 25 mL of methylene chloride, and agitate for 40 s. Centrifuge the mixture for 10 min. Use the clear upper aqueous layer.

Blank: Transfer 5 mL of *Diluent* to a 50-mL polypropylene screw-cap centrifuge tube containing 5 mL of *Solution B*, and mix for 3 min. Add 4 mL of *Solution C*, and agitate for 30 s. Allow the solution to stand at room temperature for 25 min. Add 25 mL of methylene chloride, and agitate for 40 s. Centrifuge the mixture for 10 min. Use the clear upper aqueous layer.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)