• **USP REFERENCE STANDARDS** ⟨11⟩
USP Cholecalciferol RS
USP Vitamin D Assay System Suitability RS

Cholecalciferol Solution

DEFINITION
Cholecalciferol Solution is a solution of Cholecalciferol in an edible vegetable oil, in Polysorbate 80, or in Propylene Glycol. It contains NLT 90.0% and NMT 120.0% of the labeled amount of vitamin D as cholecalciferol ($C_{27}H_{44}O$).

ASSAY
• **PROCEDURE**
Mobile phase: Hexane and pentanol (997:3)
Standard stock solution: Dissolve USP Cholecalciferol RS in toluene, and dilute with *Mobile phase* to 50 µg/mL. [NOTE—Prepare this solution fresh daily.]
Standard solution A: 5 µg/mL from *Standard stock solution* in *Mobile phase*. [NOTE—Store at a temperature not above 0°.]
Standard solution B: Transfer 5.0 mL of *Standard stock solution* to a round-bottom flask fitted with a reflux condenser. Displace the air with nitrogen, and reflux for 1 h in a water bath under a nitrogen atmosphere to obtain a solution containing cholecalciferol and precholecalciferol. Cool, transfer the solution with the aid of several portions of *Mobile phase* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.
Sample solution: Equivalent to 5 µg/mL of cholecalciferol in *Mobile phase* from an accurately measured volume of Cholecalciferol Solution
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L3
Flow rate: 2 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution B*
[NOTE—The relative retention times for precholecalciferol and cholecalciferol are about 0.4 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.0 between the precholecalciferol peak and the cholecalciferol peak
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*
Cholecalciferol response factor
Calculate the cholecalciferol response factor, F_C:

$$F_C = C_S/r_S$$

C_S = concentration of USP Cholecalciferol RS in *Standard solution A* (µg/mL)
r_S = peak area of cholecalciferol from *Standard solution A*

Pre-cholecalciferol response factor
Calculate the concentration, C'_S, in µg/mL, of cholecalciferol in *Standard solution B*:

$$C'_S = F_C \times r'_S$$

F_C = response factor for cholecalciferol
r'_S = peak area of cholecalciferol from *Standard solution B*

Calculate the concentration, C'_{pre}, in µg/mL, of precholecalciferol:

$$C'_{pre} = C_S - C'_S$$

C_S = concentration of USP Cholecalciferol RS in *Standard solution A* (µg/mL)
C'_S = concentration of cholecalciferol in *Standard solution B* (µg/mL)

Calculate the response factor, F_{pre}, for pre-cholecalciferol:

$$F_{pre} = C'_{pre}/r_p$$

C'_{pre} = concentration of pre-cholecalciferol (µg/mL)
r_p = peak response of pre-cholecalciferol from *Standard solution B*

Content of vitamin D
Calculate the percentage of the labeled amount of vitamin D as cholecalciferol ($C_{27}H_{44}O$) in the portion of the Cholecalciferol Solution taken:

$$\text{Result} = \{[(F_C \times r_C) + (F_{pre} \times r_{pre})]/C_U\} \times 100$$

F_C = response factor for cholecalciferol
r_C = peak area of cholecalciferol from the *Sample solution*
F_{pre} = response factor for pre-cholecalciferol
r_{pre} = peak area of pre-cholecalciferol from the *Sample solution*
C_U = nominal concentration of cholecalciferol in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–120.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **LABELING:** Label it to indicate the concentration, in mg/mL, of cholecalciferol. Label it also to state that it is to be used for manufacturing only.
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Cholestyramine Resin

Cholestyramine.
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» Cholestyramine Resin is a strongly basic anion-exchange resin in the chloride form, consisting of styrene-divinylbenzene copolymer with quaternary ammonium functional groups. Each g exchanges not less than 1.8 g and not more than 2.2 g of sodium glycocholate, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩**—**
USP Cholestyramine Resin RS

Identification—*Infrared Absorption* ⟨197K⟩.

pH ⟨791⟩**:** between 4.0 and 6.0, in a slurry (1 in 100).

Loss on drying ⟨731⟩**—**Dry over phosphorus pentoxide at a pressure not exceeding 50 mm of mercury at 70° for 16 hours: it loses not more than 12.0% of its weight.

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

Heavy metals, *Method II* ⟨231⟩**:** 0.002%.

Dialyzable quaternary amines—

pH 9.2 Buffer—Transfer 3.80 g of sodium borate decahydrate to a 1000-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

Bromothymol blue solution—Transfer 150 mg of bromothymol blue and 405 mg of sodium carbonate to a 100-mL volumetric flask, dilute with water to volume, and mix.

Standard solution—Take 1 mL of 60% benzyltrimethylammonium chloride solution, accurately pipeted, and dilute quan-

titatively, and stepwise, with water to obtain a stock solution having a concentration of 0.2 ± 0.01 mg per mL [NOTE—Prepare this solution fresh]. Cut a 20- to 25-cm piece of cellulose dialysis tubing* having a molecular weight cut-off that falls within the 6,000 to 14,000 range and a dry flat width of 5 to 9 cm, and place it in water to hydrate until pliable, appropriately sealing one end. Pipet 5 mL of the stock solution into the tubing, add 5 mL of water, appropriately seal the open end, place the tube in a suitable vessel containing 100 mL of water so that it is completely immersed in the water, and stir the fluid for 16 hours to effect dialysis.

Test solution—Cut a 20- to 25-cm piece of cellulose dialysis tubing* having a molecular weight cut-off that falls within the 6,000 to 14,000 range and a dry flat width of 5 to 9 cm, and place it in water to hydrate until pliable, appropriately sealing one end. Weigh 2 ± 0.01 g of Cholestyramine Resin, and carefully transfer the specimen into the tubing, taking care to ensure that none adheres to the upper walls of the tubing. Add 10 mL of water to the contents of the tube, appropriately seal the open end, and place the tube in a suitable vessel containing 100 mL of water so that it is completely immersed in the water. Stir the fluid for 16 hours to effect dialysis.

Procedure—Pipet the following into each of three separators: Separator 1—5 mL of *Standard solution,* 5 mL of *pH 9.2 Buffer,* 1 mL of *Bromothymol blue solution,* and 10 mL of chloroform; Separator 2—5 mL of *Test solution,* 5 mL of *pH 9.2 Buffer,* 1 mL of *Bromothymol blue solution,* and 10 mL of chloroform; Separator 3—5 mL of water, 5 mL of *pH 9.2 Buffer,* 1 mL of *Bromothymol blue solution,* and 10 mL of chloroform. Shake each separator vigorously for 1 minute, allow the phases to separate until the chloroform phase is clear, and collect the chloroform extracts in separate 25-mL volumetric flasks. Repeat the extraction process with a second 10-mL portion of chloroform, and combine with the previous extracts. Dilute each solution with chloroform to volume, if necessary, and mix. Concomitantly determine the absorbances of the *Test solution* and the *Standard solution* at the wavelength of maximum absorbance at about 420 nm, with a suitable spectrophotometer, using the solution from Separator 3 as the blank: the absorbance of the *Test solution* does not exceed that of the *Standard solution* (0.05% as benzyltrimethylammonium chloride).

Chloride content—To about 750 mg of Cholestyramine Resin, accurately weighed, add 100 mL of water and 50 mg of potassium nitrate. Add, with stirring, 2 mL of nitric acid, and titrate with 0.1 N silver nitrate VS, determining the endpoint potentiometrically, and using a silver-glass electrode system. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of Cl. Not less than 13.0% and not more than 17.0% of Cl, calculated on the dried basis, is found.

Exchange capacity—

Mobile phase—Prepare a filtered and degassed mixture of 0.08 M monobasic potassium phosphate and acetonitrile (65:35). Adjust with phosphoric acid to a pH of 3.0. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Potassium phosphate buffer—Transfer about 4 g of monobasic potassium phosphate and about 12 g of dibasic potassium phosphate to a 1-liter volumetric flask. Dissolve in and dilute with water to volume, and mix.

Sodium glycocholate solution—Transfer about 15 g of sodium glycocholate to a 500-mL volumetric flask, and dissolve in and dilute with *Potassium phosphate buffer* to volume.

Reference solution—Pipet 4.0 mL of *Sodium glycocholate solution* into a 100-mL volumetric flask, and dilute with water to volume.

Standard solution—Transfer about 100 mg of USP Cholestyramine Resin RS, accurately weighed, to a 25-mL conical flask. Pipet 15.0 mL of *Sodium glycocholate solution* into the flask, and stir by mechanical means for 2 hours. Transfer the contents to a centrifuge tube, and centrifuge for 15 minutes. Transfer 5.0 mL of the supernatant to a 50-mL volumetric flask, and dilute with water to volume.

System suitability solution—Prepare a solution in water containing, in each mL, about 0.6 mg of sodium glycocholate and about 0.3 mg of taurodeoxycholic acid.

Test solution—Transfer about 100 mg of anhydrous Cholestyramine Resin, accurately weighed, to a 25-mL conical flask. Pipet 15.0 mL of *Sodium glycocholate solution* into the flask, and stir by mechanical means for 2 hours. Transfer the contents to a centrifuge tube, and centrifuge for 15 minutes. Transfer 5.0 mL of the supernatant to a 50-mL volumetric flask, and dilute with water to volume.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 214-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure:* the resolution, *R,* between sodium glycocholate and taurodeoxycholic acid is not less than 1.5. Chromatograph the *Reference solution*, and record the peak responses as directed for *Procedure:* the tailing factor is not more than 2.5; and the relative standard deviation for replicate injections is not more than 1.5%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Reference solution*, the *Standard solution*, and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of sodium glycocholate absorbed on each g of the Resin taken by the formula:

$$M(2.5r_R - r_U)W_S / (2.5r_R - r_S)W_U$$

in which M is the stated value, in mg, of sodium glycocholate absorbed per g of USP Cholestyramine Resin RS; r_R, r_U, and r_S are the peak responses obtained from the *Reference solution*, the *Test solution*, and the *Standard solution*, respectively; W_U is the weight, in mg, of Cholestyramine Resin, calculated on the dried basis, taken to prepare the *Test solution;* and W_S is the weight, in mg, of USP Cholestyramine Resin RS taken to prepare the *Standard solution.*

*A suitable tubing is Spectra/Por 1, Item # 132 665, available from Spectrum Laboratories, Inc. (www.spectrapor.com), or equivalent.

Cholestyramine for Oral Suspension

» Cholestyramine for Oral Suspension is a mixture of Cholestyramine Resin with suitable excipients and coloring and flavoring agents. It contains not less than 85.0 per cent and not more than 115.0 percent of the labeled amount of dried cholestyramine resin.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩**—**
USP Cholestyramine Resin RS

Identification—Transfer a quantity of Cholestyramine for Oral Suspension, equivalent to about 500 mg of dried cholestyramine resin, to a suitable flask, add 100 mL of 0.1 N hydrochloric acid, stir to suspend the solid, and heat on a steam bath for 10 minutes. Filter, wash the residue with three 50-mL portions of water, and dry at 70° and at a pressure not exceeding 50 mm of mercury for 16 hours: the IR absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Cholestyramine Resin RS.

Uniformity of dosage units ⟨905⟩: meets the requirements for *Weight Variation.*

Assay—

Mobile phase, Potassium phosphate buffer, Sodium glycocholate solution, Reference solution, Standard solution, System suitability