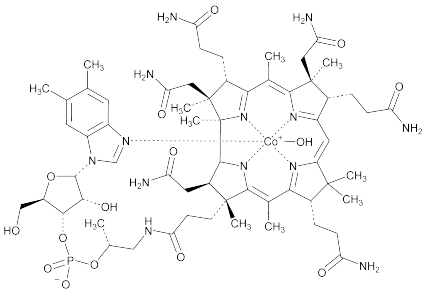
sponses for the major peaks. The retention time is about 4 minutes for hydroquinone. Calculate the quantity, in mg, of hydroquinone ($C_6H_6O_2$) in each mL of the T opical Solution taken by the formula:

$$100(C / V)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Hydroquinone RS in the *Standard preparation*; V is the volume, in mL, of Topical Solution taken; and r_U and r_S are the peak responses of hydroquinone obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Hydroxocobalamin



$C_{62}H_{89}CoN_{13}O_{15}P$ 1346.36

Cobinamide, dihydroxide, dihydrogen phosphate (ester), mono(inner salt), 3′-ester with 5,6-dimethyl-1-α-D-ribofuranosyl-1*H*-benzimidazole;

Cobinamide dihydroxide dihydrogen phosphate (ester), mono(inner salt), 3′-ester with 5,6-dimethyl-1-α-D-ribofuranosylbenzimidazole [13422-51-0].

DEFINITION

Hydroxocobalamin contains NLT 95.0% and NMT 102.0% of hydroxocobalamin ($C_{62}H_{89}CoN_{13}O_{15}P$), calculated on the dried basis.

IDENTIFICATION

- **A. Ultraviolet Absorption ⟨197U⟩**

  Wavelength range: 400–700 nm

  Sample solution: Use the *Sample solution* as directed in *pH-dependent Cobalamins*.

  Acceptance criteria: Meets the requirements in the chapter. The visible absorption spectrum of the *Sample solution* exhibits maxima at 426 ± 2, 516 ± 2, and 550 ± 2 nm.

- **B. Cobalt**

  Sample: 1 mg of Hydroxocobalamin

  Analysis: Fuse the *Sample* with 50 mg of potassium pyrosulfate in a porcelain crucible. Cool, break up the mass with a glass rod, add 3 mL of water, and boil until dissolved. Add 1 drop of phenolphthalein TS, and add 2 N sodium hydroxide dropwise until a pink color appears. Add 0.5 g of sodium acetate, 0.5 mL of 1 N acetic acid, and 0.5 mL of a 10-mg/mL solution of nitroso R salt. Add 0.5 mL of hydrochloric acid, and boil for 1 min.

  Acceptance criteria: A red or orange-red color appears immediately after the addition of nitroso R salt. The red or orange-red color persists after boiling with the addition of hydrochloric acid.

ASSAY

- **Procedure**

  Cyanocobalamin tracer reagent, Cresol–carbon tetrachloride solution, Phosphate–cyanide solution, Butanol–benzalkonium chloride solution, and **Alumina–resin column:** Prepare as directed in *Cobalamin Radiotracer Assay* ⟨371⟩.

  Standard solution: Use *Standardization* as directed in *Cobalamin Radiotracer Assay* ⟨371⟩.

  Sample solution: Transfer 40 mg of Hydroxocobalamin to a 2000-mL volumetric flask. Dissolve in and dilute with water to volume. Transfer 25.0 mL of this solution to a beaker. Add 5.0 mL of *Cyanocobalamin tracer reagent*, and proceed as directed for *Assay preparation* in *Cobalamin Radiotracer Assay* ⟨371⟩, beginning with "Add, while working under a hood, 5 mg of sodium nitrite …".

  Analysis

  Samples: *Standard solution* and *Sample solution*

  Proceed as directed for *Procedure* in *Cobalamin Radiotracer Assay* ⟨371⟩.

  Calculate the percentage of hydroxocobalmin ($C_{62}H_{89}CoN_{13}O_{15}P$) in the portion of Hydroxocobalamin taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (R_S/R_U) \times (M_{r1}/M_{r2}) \times 100$$

  A_U = absorbance of the *Sample solution* at 361 nm
  A_S = absorbance of the *Standard solution* at 361 nm
  C_S = concentration of USP Cyanocobalamin RS in the *Standard solution* (µg/mL)
  C_U = concentration of Hydroxocobalamin in the *Sample solution* (µg/mL)
  R_S = corrected average radioactivity values of the *Standard solution* (counts/min/mL)
  R_U = corrected average radioactivity values of the *Sample solution* (counts/min/mL)
  M_{r1} = molecular weight of hydroxocobalamin, 1346.36
  M_{r2} = molecular weight of cyanocobalamin, 1355.37

  Acceptance criteria: 95.0%–102.0% on the dried basis

IMPURITIES

- **Limit of Cyanocobalamin**

  Cyanocobalamin tracer reagent, Cresol–carbon tetrachloride solution, Butanol–benzalkonium chloride solution, and **Alumina–resin column:** Prepare as directed in *Cobalamin Radiotracer Assay* ⟨371⟩.

  Standard solution: Use *Standardization* as directed in *Cobalamin Radiotracer Assay* ⟨371⟩.

  Sample solution: 50 mg of Hydroxocobalamin in 25 mL of water

  Analysis: Transfer 5.0 mL of the *Sample solution* to a glass-stoppered, 50-mL centrifuge tube, and add 5.0 mL of *Cyanocobalamin tracer reagent* and 15 mL of *Cresol–carbon tetrachloride solution*. Insert the stopper, shake gently, centrifuge, carefully remove the upper, aqueous layer by aspiration, and discard the aspirated liquid. Add 25 mL of 5 N sulfuric acid, insert the stopper, shake gently, centrifuge, and remove and discard the upper, aqueous layer. Repeat the washing with additional 25-mL portions of the 5 N sulfuric acid until the acid wash is colorless (6–8 washings), and discard the acid washings. Add *Cresol–carbon tetrachloride solution* as necessary during the acid washings to maintain the volume of this phase at NL T 10 mL. Wash this solution successively with two 10-mL portions of saturated dibasic sodium phosphate solution and one 10-mL portion of water, and discard all of the aqueous washings. Proceed as directed for *Procedure* in *Cobalamin Radiotracer Assay* ⟨371⟩, beginning with "To the washed extract add 30 mL of a mixture of 2 volumes of *Butanol–benzalkonium chloride solution* and 1 volume of carbon tetrachloride". Repeat the same procedure for the *Standard solution*. Calculate the percentage of cyanocobalamin in the portion of Hydroxocobalamin taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (R_S/R_U) \times 100$$

  A_U = absorbance of the *Sample solution* at 361 nm
  A_S = absorbance of the *Standard solution* at 361 nm
  C_S = concentration of USP Cyanocobalamin RS in the *Standard solution* (µg/mL)
  C_U = concentration of Hydroxocobalamin in the *Sample solution* (µg/mL)

R_S = corrected average radioactivity values of the *Standard solution* (counts/min/mL)
R_U = corrected average radioactivity values of the *Sample solution* (counts/min/mL)
Acceptance criteria: NMT 5.0% on the dried basis

SPECIFIC TESTS
- **pH** ⟨791⟩
Sample solution: 20 mg/mL of solution
Acceptance criteria: 8.0–10.0
- **Loss on Drying** ⟨731⟩**:** Dry a sample at a pressure below 5 mm of mercury at 100° for 2 h: it loses 14.0%–18.0% of its weight.
- **pH-Dependent Cobalamins**
[NOTE—Perform the test in subdued light.]
Buffer A: Dissolve 23.8 g of sodium borate and 402 mg of boric acid in 1500 mL of water. The pH is 9.3.
Buffer B: Dissolve 2.61 g of sodium acetate and 20.5 g of sodium chloride in 5.25 mL of glacial acetic acid, and dilute with water to 1500 mL. The pH is 4.0.
Sample stock solution: Transfer 40 mg of Hydroxocobalamin into a 25-mL volumetric flask. Dissolve and dilute with carbon dioxide-free water to volume.
Sample solution A: Transfer 1.0-mL aliquot of the *Sample stock solution* to a glass-stoppered test tube. Add 3.0 mL of *Buffer A* and mix.
Sample solution B: Transfer 1.0-mL aliquot of the *Sample stock solution* to a glass-stoppered test tube. Add 3.0 mL of *Buffer B* and mix.
Instrumental conditions
(See *Spectrophotometry and Light-Scattering* ⟨851⟩.)
Mode: Visible
Analytical wavelength: 550 nm
Cell: 1 cm
Analysis
Samples: *Sample solution A* and *Sample solution B*
Determine the absorbance of *Sample solution A* against that of *Sample solution B*. Calculate the percentage of pH-dependent cobalamins, as hydroxocobalamin, in the portion of Hydroxocobalamin taken:

$$\text{Result} = A/(F \times C)$$

A = pH corrected absorbance of *Sample solution A*
F = coefficent of extinction (E 1%) of pure hydroxocobalamin in pH 9.3 buffer (100 mL · g^{-1} · cm^{-1}), 19.66
C = concentration of Hydroxocobalamin in *Sample solution A* (g/mL)
Acceptance criteria: 95.0%–102.0% on the dried basis

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight, light-resistant containers, and store in a cool place.
- **USP Reference Standards** ⟨11⟩
USP Cyanocobalamin RS

Hydroxocobalamin Injection

» Hydroxocobalamin Injection is a sterile solution of Hydroxocobalamin in Water for Injection. It contains not less than 95.0 per cent and not more than 115.0 per cent of the labeled amount of hydroxocobalamin ($C_{62}H_{89}CoN_{13}O_{15}P$).

Packaging and storage—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light.

USP Reference standards ⟨11⟩**—**
USP Cyanocobalamin RS
USP Endotoxin RS

Identification—Dilute 3.0 mL of Injection with pH 4.0 buffer (prepared by dissolving 2.61 g of sodium acetate and 20.5 g of sodium chloride in 5.25 mL of glacial acetic acid and sufficient water to make 1500 mL of solution) to 100 mL: the UV-visible absorption spectrum of this solution exhibits maxima at 352 ± 2 nm and 525 ± 2 nm. The ratio A_{352}/A_{525} is between 2.7 and 3.3.

Bacterial endotoxins ⟨85⟩**—**It contains not more than 0.4 USP Endotoxin Unit per μg of hydroxocobalamin.

pH ⟨791⟩: between 3.5 and 5.0.

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

Assay—

pH 9.3 Buffer—Dissolve 23.8 g of sodium borate and 402 mg of boric acid in sufficient water to make 1500 mL of solution, and mix.

Standard preparation—Dissolve a suitable quantity of USP Cyanocobalamin RS, accurately weighed, in *pH 9.3 Buffer* and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 30 μg per mL.

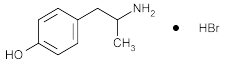
Assay preparation—Transfer an accurately measured volume of Injection, equivalent to about 5 mg of hydroxocobalamin, to a 50-mL volumetric flask containing about 25 mL of *pH 9.3 Buffer*. Add 5.0 mL of potassium cyanide solution (1 in 10,000), allow to stand at room temperature for 30 minutes, dilute with *pH 9.3 Buffer* to volume, and mix. Transfer 15.0 mL of this solution to a second 50-mL volumetric flask, dilute with *pH 9.3 Buffer* to volume, and mix.

Procedure—Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 361 nm, with a suitable spectrophotometer, using *pH 9.3 Buffer* as the blank. Calculate the quantity, in mg, of hydroxocobalamin ($C_{62}H_{89}CoN_{13}O_{15}P$) in each mL of the Injection taken by the formula:

$$(1346.36 / 1355.37)(0.1667C / V)(A_U / A_S)$$

in which 1346.36 and 1355.37 are the molecular weights of hydroxocobalamin and cyanocobalamin, respectively; C is the concentration, in μg per mL, of USP Cyanocobalamin RS in the *Standard preparation*; V is the volume, in mL, of Injection taken; and A_U and A_S are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

Hydroxyamphetamine Hydrobromide



$C_9H_{13}NO \cdot HBr$ 232.12
Phenol, 4-(2-aminopropyl)-, hydrobromide.
(±)-*p*-(2-Aminopropyl)phenol hydrobromide [306-21-8].

» Hydroxyamphetamine Hydrobromide contains not less than 98.0 per cent and not more than 101.5 percent of $C_9H_{13}NO \cdot HBr$, calculated on the dried basis.

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

USP Reference standards ⟨11⟩**—**
USP Hydroxyamphetamine Hydrobromide RS

Identification—

A: *Infrared Absorption* ⟨197K⟩.

B: Dissolve about 500 mg of ammonium molybdate in 10 mL of sulfuric acid, and add to this solution about 2 mg of Hydroxyamphetamine Hydrobromide: an intense blue color is produced *(distinction from similar amino compounds such as am-*