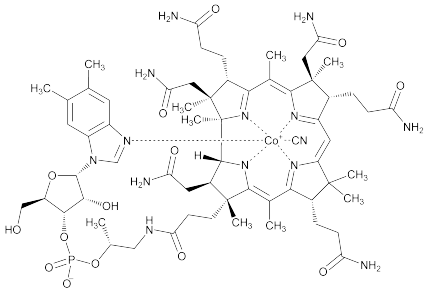
Cyanocobalamin



$C_{63}H_{88}CoN_{14}O_{14}P$ 1355.37
Vitamin B_{12} [68-19-9].

DEFINITION

Cyanocobalamin contains NLT 96.0% and NMT 102.0% of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$), calculated on the dried basis.

IDENTIFICATION

- **A. ULTRAVIOLET ABSORPTION ⟨197U⟩**
  Wavelength range: 200–700 nm
  Sample solution: Prepare as directed in the *Assay*.
  Acceptance criteria: The absorption spectrum exhibits maxima at 278 ± 1, 361 ± 1, and 550 ± 2 nm. The absorbance ratio A_{361}/A_{278} is 1.70–1.90, and the absorbance ratio A_{361}/A_{550} is 3.15–3.40.
- **B.**
  Sample solution: Fuse 1 mg of Cyanocobalamin 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 dissolve by boiling.
  Analysis: Add 1 drop of phenolphthalein TS, and add sodium hydroxide solution (100 mg/mL), dropwise, until just pink. Add 500 mg of sodium acetate, 0.5 mL of 1 N acetic acid, and 0.5 mL of nitroso R salt solution (2 mg/mL). 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 color persists after boiling with the addition of hydrochloric acid.
- **C. HPLC**
  Mobile phase and **Chromatographic system:** Proceed as directed in the test for *Related Compounds*.
  Standard solution: 50 µg/mL of cyanocobalamin from USP Cyanocobalamin RS in *Mobile phase*. Use within 1 h.
  Sample solution: 50 µg/mL of Cyanocobalamin in *Mobile phase*. Use within 1 h.
  Acceptance criteria: The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*.

ASSAY

Change to read:

- **PROCEDURE**
  ● ● (RB 1-Jun-2016)
  Sample solution: 30 µg/mL of Cyanocobalamin in water
  Instrumental conditions
  (See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)
  Mode: UV
  Analytical wavelength: 361 nm
  Cell: 1 cm
  Blank: Water
  Analysis
  ●**Sample:** *Sample solution*
  Calculate the percentage of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$) in the portion of Cyanocobalamin taken:

$$\text{Result} = A_U/(A_S \times C_U)$$

A_U = absorbance of the *Sample solution*
A_S = specific absorbance ($E^{1\%}$) of cyanocobalamin at 361 nm (100 mL · g^{-1} · cm^{-1}), 207
C_U = concentration of Cyanocobalamin in the *Sample solution* (g/mL)● (RB 1-Jun-2016)

Acceptance criteria: 96.0%–102.0% on the dried basis

IMPURITIES

- **RELATED COMPOUNDS**
  Solution A: 10 g/L of disodium hydrogen phosphate in water
  Mobile phase: Mixture of methanol and *Solution A* (26.5: 73.5). Adjust with phosphoric acid to a pH of 3.5.
  System suitability solution: Dissolve 25 mg of Cyanocobalamin in 10 mL of water, warming if necessary. Allow to cool, add 5 mL of a 1.0-g/L solution of tosylchloramide sodium and 0.5 mL of 0.05 M hydrochloric acid, and then dilute with water to 25 mL. Shake and allow to stand for 5 min. Dilute 1.0 mL of this solution with *Mobile phase* to 10 mL, and inject immediately.
  Quantitative limit solution: 1 µg/mL of Cyanocobalamin in *Mobile phase*. Use within 1 h.
  Sample solution: 1 mg/mL of Cyanocobalamin in *Mobile phase*. Use within 1 h.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 361 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L7
  Column temperature: 35°
  Flow rate: 0.8 mL/min
  Injection volume: 20 µL
  System suitability
  Samples: *System suitability solution* and *Quantitative limit solution*
  [NOTE—The *System suitability solution* should exhibit two major peaks, cyanocobalamin and 7*β*,8*β*-lactocyanocobalamin. The relative retention times for the two peaks are 1.0 and 1.2, respectively.]
  Suitability requirements
  Resolution: NLT 2.5 between cyanocobalamin and 7*β*,8*β*-lactocyanocobalamin, *System suitability solution*
  Signal-to-noise ratio: NLT 5.0 for the major peak, *Quantitative limit solution*
  Analysis
  Sample: *Sample solution*
  [NOTE—The run time should be at least three times the retention time of cyanocobalamin peak.]
  Identify the impurities listed in *Table 1*, and measure the peak responses.
  Calculate the percentage of individual impurities in the portion of Cyanocobalamin taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of each impurity from the *Sample solution*
r_T = sum of all the peak responses from the *Sample solution*

Acceptance criteria: See *Table 1*. [NOTE—Disregard any peak less than 0.1%.]

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cyanocobalamin	1.0	—
7β,8β-Lactocyanocobalamin	1.2	1.0
50-Carboxycyanocobalamin	1.4	0.5
34-Methylcyanocobalamin	1.5	2.0
32-Carboxycyanocobalamin	1.6	1.0
8-epi-Cyanocobalamin	2.5	1.0
Any other unidentified impurity	—	0.5
Total impurities	—	3.0

SPECIFIC TESTS

- **Loss on Drying** ⟨731⟩
  Sample: 25 mg
  Analysis: Dry the *Sample* in a suitable vacuum drying apparatus at 105° and at a pressure of NMT 5 mm of mercury for 2 h.
  Acceptance criteria: NMT 12.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **USP Reference Standards** ⟨11⟩
  USP Cyanocobalamin RS

Cyanocobalamin Injection

» Cyanocobalamin Injection is a sterile solution of Cyanocobalamin in Water for Injection, or in Water for Injection rendered isotonic by the addition of Sodium Chloride. It contains not less than 95.0 percent and not more than 115.0 percent of the labeled amount of anhydrous cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$).

Packaging and storage—Preserve in light-resistant, single-dose or multiple-dose containers, preferably of Type I glass, and store at controlled room temperature.

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

Identification—The absorption spectrum, in the range of 300 nm to 550 nm, of the solution employed for measurement of absorbance in the *Assay* exhibits maxima at the same wavelengths as that of a similar solution of USP Cyanocobalamin RS, concomitantly measured, and the ratio A_{361}/A_{550} is between 3.15 and 3.40.

Bacterial Endotoxins Test ⟨85⟩**—**It contains not more than 0.4 USP Endotoxin Unit per µg of cyanocobalamin.

pH ⟨791⟩**:** between 4.5 and 7.0.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—Dilute, if necessary, an accurately measured volume of Injection, equivalent to not less than 300 µg of cyanocobalamin, quantitatively and stepwise with water to a concentration of about 30 µg per mL. Dissolve an accurately weighed quantity of USP Cyanocobalamin RS in water, and dilute quantitatively and stepwise with water to obtain a Standard solution having a known concentration of about 30 µg per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 361 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in µg, of $C_{63}H_{88}CoN_{14}O_{14}P$ in each mL of the Injection taken by the formula:

$$10(C/V)(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Cyanocobalamin RS in the Standard solution; V is the volume, in mL, of Injection taken; and A_U and A_S are the absorbances of the solution from the Injection and the Standard solution, respectively.

Cyanocobalamin Tablets

DEFINITION

Cyanocobalamin Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay,Procedure 1* or *Procedure 2*.

ASSAY

[NOTE—Where more than one assay procedure is given in the monograph, the requirements may be met by following any one of the specified procedures. The procedure used is stated in the labeling only if *Procedure 1* is not used.]

- **Procedure 1**
  [NOTE—Use low-actinic glassware throughout this procedure.]
  Mobile phase: Methanol and water (7:13)
  Standard solution: 5 µg/mL of cyanocobalamin from USP Cyanocobalamin RS in water
  Sample solution: Finely powder NLT 30 Tablets. Transfer a portion of the powder, equivalent to 500 µg of cyanocobalamin, to a 100-mL volumetric flask, add 60 mL of water, and sonicate for 5 min. Dilute with water to volume, and filter.
  Chromatographic system
  (See *Chromatography* ⟨621⟩*, System Suitability*.)
  Mode: LC
  Detector: 361 nm
  Column: 4.6-mm × 15-cm; 5-µm packing L1
  Flow rate: 0.5 mL/min
  Injection volume: 100 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of the labeled amount of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$) in the portion of Tablets 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 cyanocobalamin from USP Cyanocobalamin RS in the *Standard solution* (µg/mL)
  C_U = nominal concentration of cyanocobalamin in the *Sample solution* (µg/mL)
  Acceptance criteria: 90.0%–110.0%
- **Procedure 2**
  [NOTE—Use low-actinic glassware throughout this procedure. Inject samples within 30 min.]
  Buffer: Dissolve 470.5 mg of low UV hexanesulfonic acid sodium salt in water, add 1 mL of phosphoric acid,