Cyanocobalamin

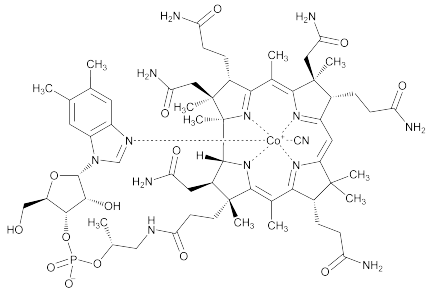
Type of Posting	Revision Bulletin
Posting Date	27–May–2016
Official Date	01–Jun–2016
Expert Committee	Non-Botanical Dietary Supplements
Reason for Revision	Compliance

In accordance with the Rules and Procedures of the 2010-2015 Council of Experts, the Non-Botanical Dietary Supplements Expert Committee has revised the *Assay* of the Cyanocobalamin monograph. The purpose for the revision is to remove the use of the *Standard solution* that has caused bias in the determination of cyanocobalamin content in the *Sample* due to its variability. The *Standard solution* is replaced with the cyanocobalamin specific absorbance for the calculation of the *Assay* results.

The Cyanocobalamin Revision Bulletin supersedes the currently official monograph. The Revision Bulletin will be incorporated in the *USP 40–NF 35*.

Should you have any questions, please contact Huy Dinh, Senior Scientific Liaison (301–816–8594 or htd@usp.org).

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⟩● (CN 1-May-2016).)
  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