<table>
<thead>
<tr>
<th><strong>Cyanocobalamin</strong></th>
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<td><strong>Type of Posting</strong></td>
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<td><strong>Expert Committee</strong></td>
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<td><strong>Reason for Revision</strong></td>
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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

C₆₃H₈₈CoN₁₄O₁₄P

Vitamin B₁₂ [68-19-9].

**DEFINITION**
Cyanocobalamin contains NLT 96.0% and NMT 102.0% of cyanocobalamin (C₆₃H₈₈CoN₁₄O₁₄P), 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 absorbance ratio A₃₆₁/A₂₇₈ is 1.70–1.90, and the absorbance ratio A₃₆₁/A₃₅₀ 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 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.

**ASSAY**

**Mode:** UV

**Analytical wavelength:** 361 nm

**Cell:** 1 cm

**Blank:** Water

**Analysis**

- **Sample: Sample solution**
  - Calculate the percentage of cyanocobalamin (C₆₃H₈₈CoN₁₄O₁₄P) in the portion of Cyanocobalamin taken:
    
  \[
  \text{Result} = \frac{A_U}{(A_S + C_I)}
  \]

  \[A_U = \text{absorbance of the Sample solution}
  \]

  \[A_S = \text{specific absorbance (ε₆₃₆₁) of cyanocobalamin at 361 nm (100 mg/mL) g⁻¹ cm⁻¹), 207}
  \]

  \[C_I = \text{concentration of Cyanocobalamin in the Sample solution (g/mL)} \]

  - **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 oxide 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.

**System suitability solution**

- **Sample:** 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°C
  - **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 7β,8β-lactocyano- cobalamin. The relative retention times for the two peaks are 1.0 and 1.2, respectively.]**

**Suitability requirements**

- **Resolution:** NLT 2.5 between cyanocobalamin and 7β,8β-lactocyano-cobalamin, 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:

    \[
    \text{Result} = \left( \frac{r_U}{r_I} \right) \times 100
    \]

    \[r_U = \text{peak response of each impurity from the Sample solution}
    \]

    \[r_I = \text{sum of all the peak responses from the Sample solution}
    \]
Acceptance criteria:  See Table 1. [NOTE—Disregard any peak less than 0.1%.

<table>
<thead>
<tr>
<th>Name</th>
<th>Relative Retention Time</th>
<th>Acceptance Criteria, NMT (%)</th>
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</thead>
<tbody>
<tr>
<td>Cyanocobalamin</td>
<td>1.0</td>
<td>—</td>
</tr>
<tr>
<td>7β,8β-Lactocya-nocobalamin</td>
<td>1.2</td>
<td>1.0</td>
</tr>
<tr>
<td>50-Carboxycya-nocobalamin</td>
<td>1.4</td>
<td>0.5</td>
</tr>
<tr>
<td>34-Methylcya-nocobalamin</td>
<td>1.5</td>
<td>2.0</td>
</tr>
<tr>
<td>32-Carboxycya-nocobalamin</td>
<td>1.6</td>
<td>1.0</td>
</tr>
<tr>
<td>8-epi-Cyanocobalamin</td>
<td>2.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Any other unidentified impurity</td>
<td>—</td>
<td>0.5</td>
</tr>
<tr>
<td>Total impurities</td>
<td>—</td>
<td>3.0</td>
</tr>
</tbody>
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**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