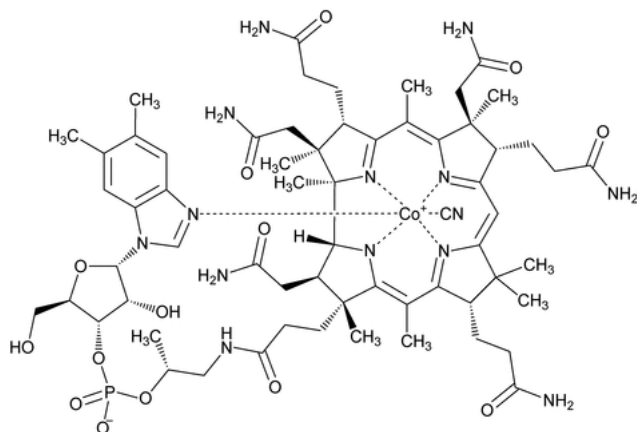


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Cyanocobalamin



$C_{63}H_{88}CoN_{14}O_{14}P$ 1355.37

Vitamin B₁₂ CAS RN®: 68-19-9; UNII: P6YC3EG204.

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. SPECTROSCOPIC IDENTIFICATION TESTS (197), Ultraviolet-Visible Spectroscopy:** 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 \(Crystalline\) 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

- **PROCEDURE**

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 ($100\text{ mL} \cdot \text{g}^{-1} \cdot \text{cm}^{-1}$), 207

C_U = concentration of Cyanocobalamin in the *Sample solution* (g/mL)

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

IMPURITIES

Change to read:

• RELATED COMPOUNDS

Solution A: 10 g/L of ▲[disodium hydrogen phosphate, dodecahydrate](#)▲ (USP 1-Dec-2023) 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 7β,8β-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 7β,8β-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 the 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	▲1.3▲ (USP 1-Dec-2023)	0.5

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
▲32-Carboxycyanocobalamin▲ (USP 1-Dec-2023)	▲1.4▲ (USP 1-Dec-2023)	2.0
▲34-Methylcyanocobalamin▲ (USP 1-Dec-2023)	▲1.5▲ (USP 1-Dec-2023)	1.0
8-epi-Cyanocobalamin	▲2.4▲ (USP 1-Dec-2023)	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 \(Crystalline\) RS](#)

Auxiliary Information - Please [check for your question in the FAQs](#) before contacting USP.

Topic/Question	Contact	Expert Committee
CYANOCOBALAMIN	Natalia Davydova Scientific Liaison	NBDS2020 Non-botanical Dietary Supplements
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	NBDS2020 Non-botanical Dietary Supplements

Chromatographic Database Information: [Chromatographic Database](#)

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