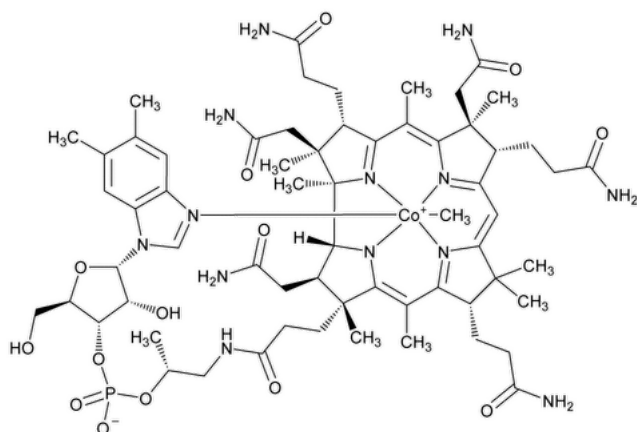


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Methylcobalamin



$C_{63}H_{91}CoN_{13}O_{14}P$ 1344.40

Co α -[α -5,6-dimethyl-1*H*-benzoimidazol-1-yl]-Co β -methylcobamide CAS RN[®]: 13422-55-4.

DEFINITION

Methylcobalamin contains NLT 98.0% and NMT 102.0% of methylcobalamin ($C_{63}H_{91}CoN_{13}O_{14}P$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

- **A.** [▲SPECTROSCOPIC IDENTIFICATION TESTS \(197\), Ultraviolet-Visible Spectroscopy: 197U](#) ▲ (CN 1-MAY-2020)

Sample solution: 50 µg/mL in phosphate buffer, pH 7.0

[NOTE—Use low-actinic glassware, and keep the solutions from exposure to light.]

Wavelength range: 200–700 nm

Acceptance criteria: The absorption spectrum exhibits maxima at 267 ± 2 nm, 342 ± 2 nm, and 522 ± 2 nm.

- **B. COBALT**

Sample: 1 mg of Methylcobalamin

Analysis: Fuse the *Sample* with 50 mg of potassium pyrosulfate in a porcelain or silica 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 2.5 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 2-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.

- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

ASSAY

PROCEDURE

Buffer solution: 3.1 g/L of sodium dihydrogen phosphate dihydrate in water. Adjust with phosphoric acid (1 in 100) to a pH of 3.5.

Mobile phase: To 200 mL of acetonitrile add 800 mL of *Buffer solution*, then add 3.76 g of sodium 1-hexane sulfonate, and mix to dissolve.

[NOTE—Use low-actinic glassware, and keep the following solutions from exposure to light.]

System suitability solution: 0.05 mg/mL each of cyanocobalamin and hydroxocobalamin acetate from [USP Cyanocobalamin \(Crystalline\) RS](#) and [USP Hydroxocobalamin Acetate RS](#) in *Mobile phase*

Standard solution: 1 mg/mL of [USP Methylcobalamin RS](#) in *Mobile phase*

Sample solution: 1 mg/mL of Methylcobalamin in *Mobile phase*

Chromatographic system

(See [Chromatography \(621\), System Suitability.](#))

Mode: LC

Detector: UV 266 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 0.6 mL/min, or adjust to have methylcobalamin elute at about 12 min.

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for cyanocobalamin and hydroxocobalamin are 0.8 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3 between the cyanocobalamin and hydroxocobalamin peaks, *System suitability solution*

Column efficiency: NLT 6000 theoretical plates, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of methylcobalamin ($C_{63}H_{91}CoN_{13}O_{14}P$) in the portion of sample 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 [USP Methylcobalamin RS](#) in the *Standard solution* (mg/mL)

C_U = concentration of Methylcobalamin in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

• RELATED COMPOUNDS

Buffer solution, Mobile phase, System suitability solution, and Chromatographic system: Proceed as directed in the Assay except for the *Run time*.

[NOTE—Use low-actinic glassware, and keep the following solutions from exposure to light.]

Sample solution: 1 mg/mL of Methylcobalamin in *Mobile phase*

Quantitative limit solution: 1 µg/mL of Methylcobalamin in *Mobile phase*, diluted from the *Sample solution*

Run time: NLT 2.5 times the retention time of methylcobalamin

System suitability

Samples: *System suitability solution* and *Quantitative limit solution*

Suitability requirements

Resolution: NLT 3 between the cyanocobalamin and hydroxocobalamin peaks, *System suitability solution*

Signal-to-noise ratio: NLT 5.0 for the major peak, *Quantitative limit solution*

Analysis

Sample: *Sample solution*

Calculate the percentage of individual impurities in the portion of Methylcobalamin 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

Individual impurities: NMT 0.5%

Total impurities: NMT 2.0%

SPECIFIC TESTS

- [WATER DETERMINATION \(921\)](#), [Method I](#), [Method Ia](#): 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](#)

[USP Hydroxocobalamin Acetate RS](#)

[USP Methylcobalamin RS](#)