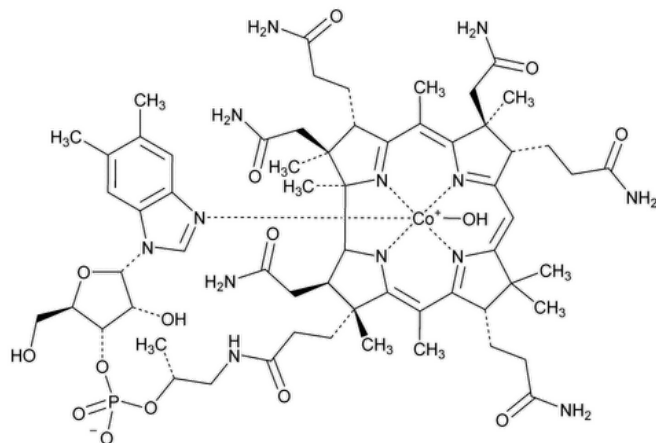


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Hydroxocobalamin



$C_{62}H_{89}CoN_{13}O_{15}P$ 1346.36

Cobinamide, dihydroxide, dihydrogen phosphate (ester), mono(inner salt), 3'-ester with 5,6-dimethyl-1- α -D-ribofuranosyl-1H-benzimidazole;
 Cobinamide dihydroxide dihydrogen phosphate (ester), mono(inner salt), 3'-ester with 5,6-dimethyl-1- α -D-ribofuranosylbenzimidazole CAS
 RN®: 13422-51-0; UNII: Q40X8H422O.

DEFINITION

Hydroxocobalamin contains NLT 95% and NMT 102.0% of hydroxocobalamin ($C_{62}H_{89}CoN_{13}O_{15}P$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

Change to read:

• **A.** [▲ SPECTROSCOPIC IDENTIFICATION TESTS \(197\), Infrared Spectroscopy: 197K ▲](#) (CN 1-MAY-2020)

• B. COBALT

Sample: 1 mg of Hydroxocobalamin

Analysis: Fuse the *Sample* 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 boil until dissolved. Add 1 drop of phenolphthalein TS, and add 2 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 10-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: 15.6 g/L of sodium dihydrogen phosphate in water. Adjust with phosphoric acid (1 in 100) to a pH of 3.0.

Mobile phase: See [Table 1](#).

Table 1

Time (min)	Methanol (%)	Buffer solution (%)	Water (%)
0	8	10	82
20	8	10	82
40	40	10	50
45	8	10	82
50	8	10	82

Diluent: Methanol, Buffer solution, and water (8:10:82)

Standard solution: 0.1 mg/mL of [USP Hydroxocobalamin Chloride RS](#) in Diluent. Mix to homogenize.

Sample solution: 0.1 mg/mL of Hydroxocobalamin in Diluent. Mix to homogenize.

Chromatographic system

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

Mode: LC

Detector: UV 351 nm

Column: 4.6-mm × 10-cm and 4.6-mm × 10-cm placed in series; packing L1¹

Column temperature: 30°

Flow rate: 2.0 mL/min

Injection volume: 20 µL

System suitability

Sample: Standard solution

[NOTE—The retention time of hydroxocobalamin is about 16 min.]

Suitability requirements

Tailing factor: NMT 2.5

Relative standard deviation: NMT 1.0% from replicate injections

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of hydroxocobalamin ($C_{62}H_{89}CoN_{13}O_{15}P$) in the portion of Hydroxocobalamin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response from the Sample solution

r_S = peak response from the Standard solution

C_S = concentration of [USP Hydroxocobalamin Chloride RS](#) in the Standard solution (mg/mL)

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

M_{r1} = molecular weight of hydroxocobalamin, 1346.4

M_{r2} = molecular weight of hydroxocobalamin chloride, 1382.8

Acceptance criteria: 95%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

• RELATED COMPOUNDS

Buffer solution, Mobile phase, Diluent, and Chromatographic system: Proceed as directed in Assay.

System suitability solution: 0.75 mg/mL of [USP Hydroxocobalamin Chloride RS](#) in Diluent

Solution for quantitation: 7.5 µg/mL of [USP Hydroxocobalamin Chloride RS](#) in Diluent, diluted from the System suitability solution

Solution for the disregard limit: 0.75 µg/mL of [USP Hydroxocobalamin Chloride RS](#) in Diluent, diluted from the Solution for quantitation

Sample solution: 0.75 mg/mL of Hydroxocobalamin in Diluent