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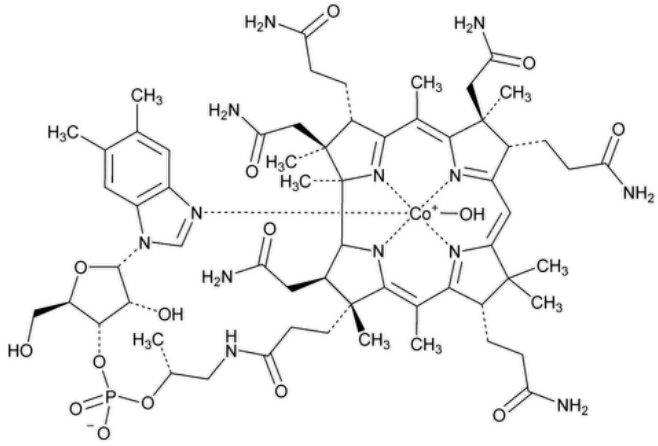
Hydroxocobalamin



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

Cobinamide, dihydroxide, dihydrogen phosphate (ester), mono(inner salt), 3'-ester with 5,6-dimethyl-1-α-D-ribofuranosyl-1*H*-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 L1[1]

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*

System suitability

Sample: *System suitability solution*

Suitability requirement

Peak-to-valley ratio:[2] NLT 2.0 between hydroxocobalamin and its closest impurity

Analysis

Sample: *Sample solution*

Measure the peak responses of the *Sample solution*. Disregard the peaks with a response smaller than 0.7 times that of the peak of the *Solution for the disregard limit*.

Calculate the percentage of each impurity in the portion of Hydroxocobalamin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_S = peak response from the *Solution for quantitation*

C_S = concentration of USP Hydroxocobalamin Chloride RS in the *Solution for quantitation*

C_U = concentration of Hydroxocobalamin in the *Sample solution*

F = response factor, 0.8 for cyanocobalamin and 1.0 for any other impurity; *Sample solution*

M_{r1} = molecular weight of hydroxocobalamin, 1346.4

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

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
B6-Hydroxymethyl derivative	0.45	0.50
B5-Hydroxymethyl derivative	0.53	0.40
Impurity	0.70	0.30
Impurity	0.75	0.40
Impurity	0.82	0.40
Impurity	0.93	1.1
C8 Epimer	1.25	1.0
Impurity	1.37–1.51	0.50
Cyanocobalamin	1.90	1.0
Any other impurity	—	0.20
Sum of all impurities	—	4.0

- **Residual Solvents ⟨467⟩**

Acceptance criteria

Acetone: NMT 0.5%. [Note—For *Acceptance criteria* for any other residual solvents, see the chapter.]

SPECIFIC TESTS

• **pH** ⟨791⟩

Sample solution: 20 mg/mL of solution

Acceptance criteria: 8.0–10.0

• **Water Determination,** *Method Ia*⟨921⟩**:** 14.0%–18.0%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight, light-resistant containers, and store in a cool, dry place. Do not freeze.

• **USP Reference Standards** ⟨11⟩

USP Hydroxocobalamin Chloride RS

[1] Chromolith columns (Merck) are based on a reversed phase employing monolithic C_{18}-bonded silica with bimodal pore structure (macropore 2 µm; mesopore 13 nm). Example: Merck Chromolith Performance RP-18 endcapped, 4.6-mm × 10-cm, product # 1021290001.

[2] Peak-to-valley ratio:

$$p/v = H_P/H_v$$

p/v = peak-to-valley ratio

H_P = height above the extrapolated baseline of the minor peak

H_v = height above the extrapolated baseline at the lowest point of the curve separating the minor and major peaks

(See Chromatography ⟨621⟩, Peak-to-Valley Ratio.) NLT 2.0 between hydroxocobalamin and its closest impurity

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
HYDROXOCOBALAMIN	Natalia Davydova Scientific Liaison	NBDS2020 Non-botanical Dietary Supplements

Chromatographic Database Information: Chromatographic Database

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