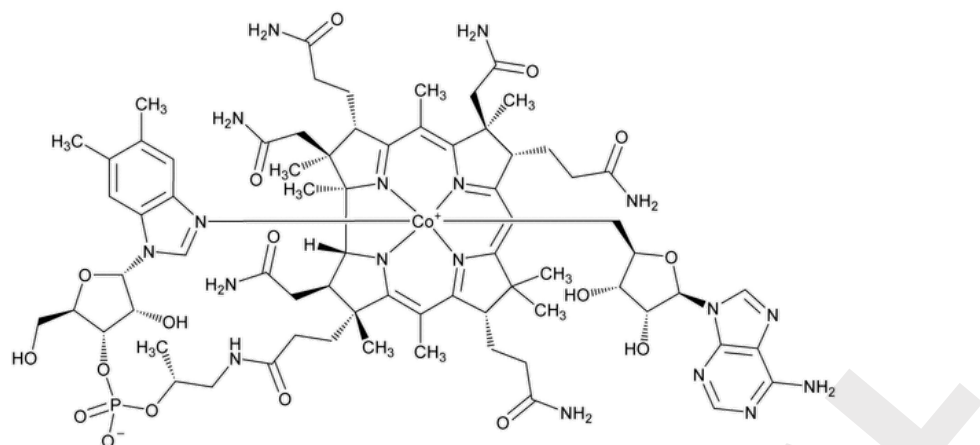


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Add the following:

▲Cobamamide



$C_{72}H_{100}CoN_{18}O_{17}P$ 1579.58
 5,6-Dimethylbenzimidazolyl-Co-5'-deoxyadenosylcobamide;
 Adenosylcobalamin CAS RN®: 13870-90-1.

DEFINITION

Cobamamide contains NLT 96.0% and NMT 102.0% of cobamamide ($C_{72}H_{100}CoN_{18}O_{17}P$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. SPECTROSCOPIC IDENTIFICATION TESTS (197), Infrared Spectroscopy: (197K).**
- **B. SPECTROSCOPIC IDENTIFICATION TESTS (197), Ultraviolet-Visible Spectroscopy: (197U).**

[NOTE—Use low-actinic glassware, and protect the *Sample solution* from exposure to light.]

Wavelength range: 200–700 nm

Buffer solution: Dissolve 6.8 g of [monobasic potassium phosphate](#) in 950 mL of [water](#). Adjust with 50% [sodium hydroxide](#) solution to a pH of 7.0. Dilute with [water](#) to 1000 mL.

Sample solution: 50 µg/mL in *Buffer solution*

Acceptance criteria: The absorption spectrum exhibits maxima at 260, 288, 376, and 524 nm. The absorbance ratio A_{260}/A_{524} is 4.1–4.5; the absorbance ratio A_{376}/A_{260} is 0.29–0.33.

- **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

Solution A: [Methanol](#)

Solution B: Dissolve 6.8 g of [monobasic potassium phosphate](#) in 950 mL of [water](#). Adjust with 10% phosphoric acid to a pH of 3.2. Dilute with [water](#) to 1000 mL. Pass this solution through a filter of 0.2-µm pore size.

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

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	15	85
2	15	85
2.5	20	80
8	23	77
10	25	75
22	30	70
24	35	65
30	35	65
31	70	30
40	70	30
40.5	15	85
45	15	85

Diluent: [Methanol](#) and [water](#) (1:3)

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

System suitability solution: 0.05 mg/mL each of [USP Cobamamide RS](#), [USP Cyanocobalamin \(Crystalline\) RS](#), [USP Hydroxocobalamin Chloride RS](#), and [USP Methylcobalamin RS](#) in *Diluent*

Standard solution: Allow an appropriate portion of the [USP Cobamamide RS](#) to equilibrate at room relative humidity and protected from light, until its weight shows no changes. The equilibration time may be up to 60 min or more depending on the room relative humidity. Use the equilibrated material to prepare a 1-mg/mL solution in *Diluent*. Concurrently determine the water content of the equilibrated sample and use it for the Assay correction.

Sample solution: Prepare as directed in the *Standard solution*, except replace [USP Cobamamide RS](#) with Cobamamide.

Chromatographic system

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

Mode: LC

Detector: UV 260 nm

Column: 4.6-mm × 10-cm; 2.6-μm packing L1

Column temperature: 40°

Flow rate: 1.2 mL/min

Injection volume: 5 μL

System suitability

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

[NOTE—The relative retention times for cobamamide, hydroxocobalamin, cyanocobalamin, and methylcobalamin are 1.00, 0.15, 0.40, and 1.47, respectively, *System suitability solution*.]

Suitability requirements

Column efficiency: NLT 22,000 theoretical plates for the cobamamide peak, *Standard solution*

Tailing factor: NMT 2.0 for the cobamamide peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of cobamamide ($C_{72}H_{100}CoN_{18}O_{17}P$) in the portion of Cobamamide taken:

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