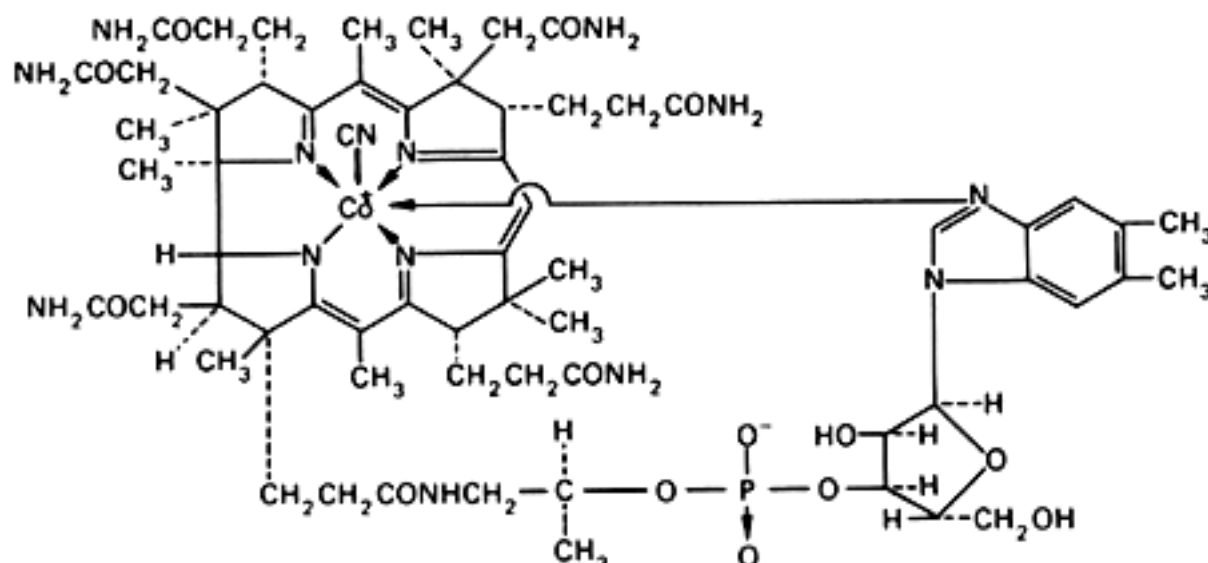


**Cyanocobalamin (Cyanocobalaminum)****Molecular formula.**  $C_{63}H_{88}CoN_{14}O_{14}P$ **Relative molecular mass.** 1355**Graphic formula.****Chemical name.**  $\alpha$ -(5,6-Dimethylbenzimidazol-2-yl)cobamide cyanide; CAS Reg. No. 68-19-9.**Other name.** Vitamin B<sub>12</sub>.**Description.** Dark red crystals or a red, crystalline powder; odourless.**Solubility.** Soluble in 80 parts of water; soluble in ethanol (~750 g/l) TS; practically insoluble in acetone R and ether R.**Category.** Haemopoietic.**Storage.** Cyanocobalamin should be kept in a tightly closed container, protected from light.**Additional information.** The anhydrous form of Cyanocobalamin is highly hygroscopic.**Requirements****Definition.** Cyanocobalamin contains not less than 96.0% and not more than 102.0% of  $C_{63}H_{88}CoN_{14}O_{14}P$ , calculated with reference to the dried substance.**Identity tests**

A. The absorption spectrum of a 20  $\mu$ g/mL solution, when observed between 230 nm and 600 nm, exhibits 3 maxima at about 278 nm, 361 nm and 550 nm; the ratio of the absorbance of a 1-cm layer at 361 nm to that at 278 nm is between 1.70 and 1.90, and the ratio of the absorbance at 361 nm to that at 550 nm is between 3.15 and 3.45.

B. Mix 1 mg with about 10 mg of potassium sulfate R and 2 drops of sulfuric acid (~100 g/l) TS, heat the mixture carefully to redness until fused. Cool, break up the mass with a glass rod, and dissolve in 3 mL of water by boiling. Add 1 drop of phenolphthalein/ethanol TS and, drop by drop, sodium hydroxide (~80 g/l) TS until the solution is just pink. Add 0.5 g of sodium acetate R, 0.5 mL of acetic acid (~60 g/l) TS, and 0.5 mL of 1-nitroso-2-naphthol-3,6-disodium disulfonate (2 g/l) TS; a red or orange-red colour appears immediately. Add 0.5 mL of hydrochloric acid (~250 g/l) TS and boil for 1 minute; the red colour persists.

**Clarity of solution.** A solution of 20 mg in 10 mL of water is clear.**Loss on drying.** Dry to constant weight at 105°C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury); it loses not more than 120 mg/g.

**Pseudocyanocobalamin.** Dissolve 1 mg in 20 mL of water. Transfer the solution to a small separator, add 5 mL of a mixture of equal volumes of carbon tetrachloride R and freshly distilled *o*-cresol R, and shake well for about 1 minute. Allow to separate, draw off the lower layer into a second small separator, add a mixture of 2.5 mL of sulfuric acid (~570 g/l) TS and 2.5 mL of water, shake well and allow to separate completely (the separation of the layers may be facilitated by centrifuging). Prepare a reference solution containing 1.5 mL of potassium permanganate (0.002 mol/l) VS in 250 mL of water. The separated upper layer of the test

solution is colourless or not more intensely coloured than the reference solution when compared as described under [1.11 Colour of liquids](#).

**Related substances.** Carry out the test as described under [1.14.1 Thin-layer chromatography](#), using equal parts of silica gel R1 and kieselguhr R1 as the coating substance and a mixture of 15 volumes of chloroform R, 10 volumes of methanol R, and 3 volumes of ammonia (~100 g/l) TS as the mobile phase. Carry out all operations protected from light. Apply separately to the plate 10 µl of each of 3 solutions containing (A) 5.0 mg of the test substance per mL, (B) 0.20 mg of the test substance per mL, and (C) 0.10 mg of the test substance per mL. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in daylight. Any spot obtained with solution A, other than the principal spot is not more intense than that obtained with solution B. Not more than one spot obtained with solution A, other than the principal spot, is more intense than that obtained with solution C.

**Assay.** Dissolve about 0.03 g, accurately weighed, in sufficient water to produce 1000 mL. Determine the absorbance of this solution in a 1-cm layer at the maximum at about 361 nm and calculate the content of  $C_{63}H_{88}CoN_{14}O_{14}P$ , using the absorptivity

value of 20.7 ( $\frac{A_{1\%}^{1\text{cm}}}{1\text{cm}} = 207$ ).