(4) Calcium—Dissolve 0.2 g of Cyanamide in 20 mL of water, add 2 mL of ammonia TS and 2 mL of ammonium oxalate TS, and allow to stand for 5 minutes: no turbidity is produced.

(5) Diethyl ether-insoluble substances—Dissolve 1.0 g of Cyanamide in 3.0 mL of diethyl ether by shaking, allow to stand for 10 minutes, and shake again: the solution is clear.

Water Not more than 1.0% (1 g, direct titration).

Residue on ignition Not more than 0.10% (1 g).

Assay Weigh accurately about 1 g of Cyanamide, and dissolve in water to make exactly 250 mL. Pipet 15 mL of this solution, add 2 to 3 drops of dilute nitric acid, 10 mL of ammonia TS and exactly 50 mL of 0.1 mol/L silver nitrate VS, and allow to stand for 15 minutes with occasional shaking. Add water to make exactly 100 mL, filter, discard the first 20 mL of the filtrate, and pipet the subsequent 50 mL. After neutralizing this solution with dilute nitric acid, add 3 mL of dilute nitric acid, and titrate the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS). Perform a blank determination.

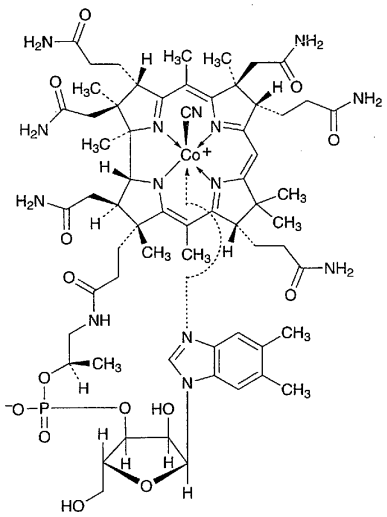
Each mL of 0.1 mol/L silver nitrate VS
= 2.1020 mg of CH_2N_2

Containers and storage Containers—Tight containers.
Storage—In a cold place.

Cyanocobalamin

Vitamin B_{12}

シアノコバラミン



$C_{63}H_{88}CoN_{14}O_{14}P$: 1355.37
Coα-[α-(5,6-Dimethylbenz-1*H*-imidazol-1-yl)]-*Coβ*-cyanocobamide [*68-19-9*]

Cyanocobalamin contains not less than 95.0% of $C_{63}H_{88}CoN_{14}O_{14}P$, calculated on the dried basis.

Description Cyanocobalamin occurs as dark red crystals or powder.

It is sparingly soluble in water, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

It is hygroscopic.

Identification **(1)** Determine the absorption spectrum of the sample solution obtained in the Assay as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cyanocobalamin Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Mix 1 mg of Cyanocobalamin with 0.05 g of potassium hydrogen sulfate, and fuse by igniting. Cool, break up the mass with a glass rod, add 3 mL of water, and dissolve by boiling. Add 1 drop of phenolphthalein TS, then add dropwise sodium hydroxide TS until a light red color just develops. Add 0.5 g of sodium acetate trihydrate, 0.5 mL of dilute acetic acid and 0.5 mL of a solution of disodium 1-nitroso-2-naphthol-3,6-disulfonate (1 in 500): a red to orange-red color is immediately produced. Then add 0.5 mL of hydrochloric acid, and boil for 1 minute: the red color does not disappear.

(3) Transfer 5 mg of Cyanocobalamin to a 50-mL distilling flask, dissolve in 5 mL of water, and add 2.5 mL of hypophosphorous acid. Connect the flask with a short condenser, and dips its tip into a test tube containing 1 mL of a solution of sodium hydroxide (1 in 50). Heat gently for 10 minutes, then distil 1 mL into a test tube. To the test tube add 4 drops of a saturated solution of ammonium iron (II) sulfate hexahydrate, shake gently, then add about 0.03 g of sodium fluoride, and heat the contents to boil. Immediately add dropwise diluted sulfuric acid (1 in 7) until a clear solution results, then add 3 to 5 drops more of diluted sulfuric acid (1 in 7): a blue to blue-green color develops.

pH Dissolve 0.10 g of Cyanocobalamin in 20 mL of water: the pH of this solution is between 4.2 and 7.0.

Purity **(1)** Clarity and color of solution—Dissolve 0.020 g of Cyanocobalamin in 10 mL of water: the solution is clear and red in color.

(2) Pseudocyanocobalamin—Dissolve 1.0 mg of Cyanocobalamin in 20 mL of water, transfer the solution to a separator, add 5 mL of a mixture of *m*-cresol and carbon tetrachloride (1:1), and shake vigorously for 1 minute. Allow to separate, draw off the lower layer into another separator, add 5 mL of diluted sulfuric acid (1 in 7), shake vigorously, and allow to separate completely. If necessary, centrifuge the mixture: the supernatant liquid is colorless or has no more color than the following control solution.

Control solution: Dilute 0.6 mL of 0.02 mol/L potassium permanganate VS with water to make 1000 mL.

Loss on drying Not more than 12% (0.05 g, in vacuum at a pressure not exceeding 0.67 kPa, phosphorus (V) oxide, 100°C, 4 hours).

Assay Weigh accurately about 0.02 g each of Cyanocobalamin and Cyanocobalamin Reference Standard (previously determine the loss on drying in the same manner as Cyanocobalamin), dissolve in water to make exactly 1000 mL, respectively, and use these solutions as the sample solution and the standard solution. Determine the absorbances,

A_T and A_S, of the sample solution and the standard solution, respectively, at 361 nm as directed under the Ultraviolet-visible Spectrophotometry.

Amount (mg) of $C_{63}H_{88}CoN_{14}O_{14}P$
= amount (mg) of Cyanocobalamin Reference Standard, calculated on the dried basis
$\times \frac{A_T}{A_S}$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Cyanocobalamin Injection

Vitamin B_{12} Injection

シアノコバラミン注射液

Cyanocobalamin Injection is an aqueous solution for injection. It contains not less than 95% and not more than 115% of the labeled amount of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$: 1355.37)

Method of preparation Prepare as directed under Injections, with Cyanocobalamin.

Description Cyanocobalamin Injection is a clear, light red to red liquid
It is gradually affected by light.
pH: 4.0 – 5.5

Identification Determine the absorption spectrum of the sample solution obtained in the Assay as directed under the Ultraviolet-visible Spectrophotometry: it exhibits maxima between 277 nm and 279 nm, between 360 nm, and 362 nm and between 548 nm and 552 nm. Determine the absorbances, A_1 and A_2, of this solution at the wavelengths of maximum absorption between 360 nm and 362 nm, and between 548 nm and 552 nm, respectively: the ratio A_2/A_1 is not less than 0.29 and not more than 0.32.

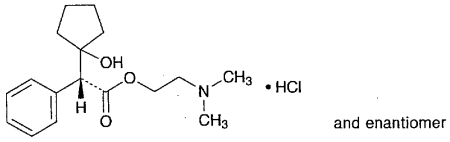
Assay Measure exactly a volume of Cyanocobalamin Injection, equivalent to about 2 mg of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$), add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.02 g of Cyanocobalamin Reference Standard (previously determine the loss on drying in the same manner as Cyanocobalamin), add water to make exactly 1000 mL, and use this solution as the standard solution. With these solutions, proceed as directed in the Assay under Cyanocobalamin.

Amount (mg) of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$)
= amount (mg) of Cyanocobalamin Reference Standard, calculated on the dried basis
$\times \frac{A_T}{A_S} \times \frac{1}{10}$

Containers and storage Containers—Hermetic containers, and colored containers may be used.
Storage—Light-resistant.

Cyclopentolate Hydrochloride

塩酸シクロペントラート



and enantiomer

$C_{17}H_{25}NO_3$.HCl: 327.85
2-(Dimethylamino)ethyl (*RS*)-(1-hydroxycyclopentyl)-phenylacetate monohydrochloride [*5870-29-1*]

Cyclopentolate Hydrochloride, when dried, contains not less than 98.5% of $C_{17}H_{25}NO_3$.HCl.

Description Cyclopentolate Hydrochloride occurs as a white, crystalline powder. It is odorless, or has a characteristic odor.

It is very soluble in water, freely soluble in ethanol (95), in acetic acid (100) and in chloroform, sparingly soluble in acetic anhydride, and practically insoluble in diethyl ether.

Identification (1) To 1 mL of a solution of Cyclopentolate Hydrochloride (1 in 100) add 1 mL of Reinecke salt TS: a light red precipitate is formed.

(2) Dissolve 0.2 g of Cyclopentolate Hydrochloride in 2 mL of water, add 2 mL of sodium hydroxide TS, and boil for 1 minute. After cooling, add 2 drops of nitric acid: a phenylacetic acid-like odor is perceptible.

(3) Determine the infrared absorption spectrum of Cyclopentolate Hydrochloride, previously dried, as directed in the potassium chloride disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Cyclopentolate Hydrochloride (1 in 50) responds to the Qualitative Tests for chloride.

pH Dissolve 0.20 g of Cyclopentolate Hydrochloride in 20 mL of water: the pH of this solution is between 4.5 and 5.5.

Melting point 135 – 138°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Cyclopentolate Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals—Proceed with 1.0 g of Cyclopentolate Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.20 g of Cyclopentolate Hydrochloride in 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add chloroform to make exactly 20 mL. Pipet 1 mL of this solution, add chloroform to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 2-propanol, *n*-butyl acetate, water and ammonia solution (28)