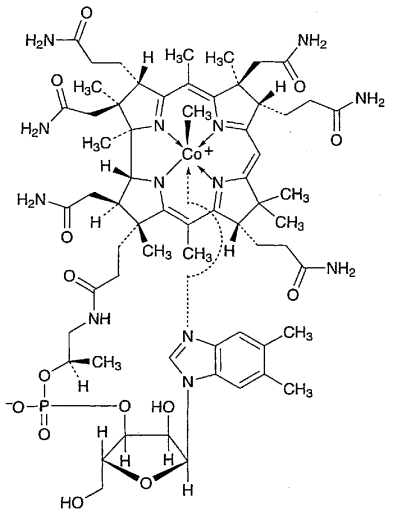
the solution changes from blue-green through yellow-green to pale greenish yellow [indicator: 3 drops of a solution of malachite green oxalate in acetic acid (100) (1 in 100)]. Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 29.418 mg of $C_{12}H_{16}ClNO_3.HCl$

Containers and storage Containers—Tight containers.

Mecobalamin

メコバラミン



$C_{63}H_{91}CoN_{13}O_{14}P$: 1344.38
Coα-[α-(5,6-Dimethylbenz-1*H*-imidazolyl)]-*Coβ*-methylcobamide [*13422-55-4*]

Mecobalamin contains not less than 98.0% of $C_{63}H_{91}CoN_{13}O_{14}P$, calculated on the anhydrous basis.

Description Mecobalamin occurs as dark red crystals or crystalline powder.

It is sparingly soluble in water, slightly soluble in ethanol (99.5), and practically insoluble in acetonitrile.

It is affected by light.

Identification (1) Conduct this procedure without exposure to light, using light-resistant vessels. Determine the absorption spectrum of a solution of Mecobalamin in hydrochloric acid-potassium chloride buffer solution, pH 2.0 (1 in 20,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum 1 or the spectrum of a solution of Mecobalamin Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, determine the absorption spectrum of a solution of Mecobalamin in phosphate buffer solution, pH 7.0 (1 in 20,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum 2 or the spectrum of a solution of Mecobalamin 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 Mecobalamin with 0.05 g of potassium bisulfate, 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, 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.

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

(2) Related substances—Perform the test with 10 μL of the sample solution obtained in the Assay as directed under the Liquid Chromatography according to the following conditions. Determine the peak area of mecobalamin and others of the sample solution by the automatic integration method: each area of the peaks other than mecobalamin is not larger than 0.5% of the peak area of mecobalamin, and the total area of the peaks other than mecobalamin is not larger than 2.0%.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2.5 times as long as the retention time of mecobalamin.

System suitability—

Test for required detection: To exactly 1 mL of the sample solution add the mobile phase to make exactly 100 mL, and use this solution as the test solution for system suitability. Pipet 1 mL of the test solution for system suitability, add the mobile phase to make exactly 10 mL. Confirm that the peak area of mecobalamin obtained from 10 μL of this solution is equivalent to 7 to 13% of that of mecobalamin obtained from 10 μL of the test solution for system suitability.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 10 μL of the test solution for system suitability under the above operating conditions, the relative standard deviation of the peak areas of mecobalamin is not more than 3.0%.

Water Not more than 12% (0.1 g, direct titration).

Assay Conduct this procedure without exposure to light, using light-resistant vessels. Weigh accurately about 0.05 g of Mecobalamin and Mecobalamin Reference Standard (separately, determine the water in the same manner as mecobalamin), dissolve each in the mobile phase to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL of each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of mecobalamin in each solution.

Amount (mg) of $C_{63}H_{91}CoN_{13}O_{14}P$
= amount (mg) of Mecobalamin Reference Standard, calculated on the anhydrous basis $\times \frac{A_T}{A_S}$

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 266 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 200 mL of acetonitrile add 800 mL of 0.02 mol/L phosphate buffer solution, pH 3.5, then add 3.76 g of sodium 1-hexane sulfonate to dissolve.

Flow rate: Adjust the flow rate so that the retention time of mecobalamin is about 12 minutes.

System suitability—

System performance: Dissolve 5 mg each of cyanocobalamin and hydroxocobalamin acetate in the mobile phase to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, cyanocobalamin and hydroxocobalamin are eluted in this order with the resolution between these peaks being not less than 3. And when the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates of the peak of mecobalamin is not less than 6000.

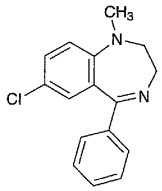
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of mecobalamin is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Medazepam

メダゼパム



$C_{16}H_{15}ClN_2$: 270.76

7-Chloro-2,3-dihydro-1-methyl-5-phenyl-1*H*-1,4-benzodiazepine [*2898-12-6*]

Medazepam, when dried, contains not less than 98.5% of $C_{16}H_{15}ClN_2$.

Description Medazepam occurs as white to light yellow crystals or crystalline powder. It is odorless.

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

It gradually changes in color by light.

Identification (1) Dissolve 0.01 g of Medazepam in 3 mL of citric acid-acetic acid TS: a deep orange color develops. Heat in a water bath for 3 minutes: the color changes to dark red.

(2) Determine the absorption spectrum of a solution of Medazepam in methanol (1 in 100,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Perform the test with Medazepam as directed under the Flame Coloration Test (2): a green color is produced.

Melting point 101 - 104°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Medazepam in 10 mL of methanol: the solution is clear and light yellow to yellow in color.

(2) Chloride—Dissolve 1.5 g of Medazepam in 50 mL of diethyl ether, add 46 mL of water and 4 mL of sodium carbonate TS, shake, and collect the water layer. Wash the water layer with two 20-mL portions of diethyl ether, and filter. To 20 mL of the filtrate add dilute nitric acid to neutralize, add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.018%).

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

(4) Arsenic—Prepare the test solution with 1.0 g of Medazepam according to Method 3, and perform the test using Apparatus B (not more than 2 ppm).

(5) Related substances—Dissolve 0.25 g of Medazepam in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 20 mL. Pipet 2 mL of this solution, add methanol to make exactly 50 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 with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of cyclohexane, acetone and ammonia solution (28) (60:40:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying Not more than 0.5% (1 g, in vacuum, 60°C, 4 hours).

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

Assay Weigh accurately about 0.4 g of Medazepam, previously dried, dissolve in 50 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 27.076 mg of $C_{16}H_{15}ClN_2$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.