pare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium—Perform the test with 0.25 g of L-Valine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals—Proceed with 1.0 g of L-Valine 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).

(6) Arsenic—Proceed with 1.0 g of L-Valine, prepare the test solution according to Method 2, and perform the test using Apparatus B (not more than 2 ppm).

(7) Other amino acids—Dissolve 0.10 g of L-Valine in 25 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 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 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 80°C for 5 minutes: 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 0.30% (1 g, 105°C, 3 hours).

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

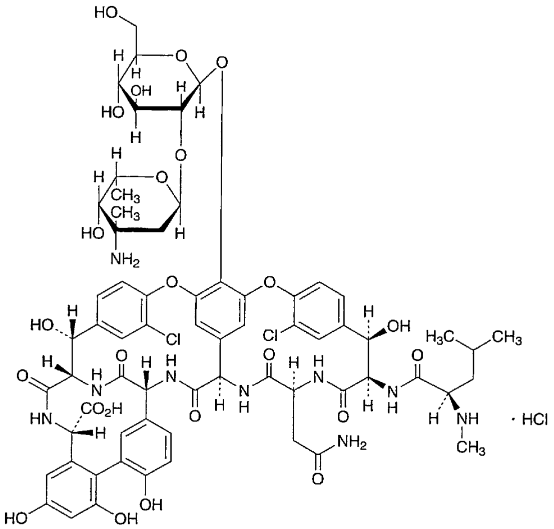
Assay Weigh accurately about 0.12 g of L-Valine, previously dried, and dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 11.715 mg of $C_5H_{11}NO_2$

Containers and storage Containers—Tight containers.

Vancomycin Hydrochloride

塩酸バンコマイシン



$C_{66}H_{75}Cl_2N_9O_{24}$.HCl: 1485.71
(1*S*,2*R*,18*R*,19*R*,22*S*,25*R*,28*R*,40*S*)-50-[2-*O*-(3-Amino-2,3,6-trideoxy-3-*C*-methyl-α-L-*lyxo*-hexopyranosyl)-β-D-glucopyranosyloxy]-22-carbamoylmethyl-5,15-dichloro-2,18,32,35,37-pentahydroxy-19-[(2*R*)-4-methyl-2-(methylamino)pentanoylamino]-20,23,26,42,44-pentaoxo-7,13-dioxa-21,24,27,41,43-pentaazaoctacyclo-$[26.14.2.2^{3,6}.2^{14,17}.1^{8,12}.1^{29,33}.0^{10,25}.0^{34,39}]$pentaconta-3,5,8,10,12(50),14,16,29,31,33(49),34,36,38,45,47-pentadecaene-40-carboxylic acid monohydrochloride
[*1404-93-9*]

Vancomycin Hydrochloride conforms to the requirements of Vancomycin Hydrochloride in the Requirements for Antibiotic Products of Japan.

Description Vancomycin Hydrochloride occurs as a white powder.

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

Vasopressin Injection

バソプレシン注射液

Vasopressin Injection is an aqueous solution for injection. It contains synthetic vasopressin or the pressor principle, vasopressin, obtained from the posterior lobe of the pituitary of healthy cattles and pigs, from which the majority of the oxytocic principle, oxytocin, has been removed.

It contains not less than 85% and not more than 120% of the labeled vasopressin Units.

Method of preparation Prepare as directed under Injections, with vasopressin prepared by synthesis or obtained from the posterior lobe of the pituitary.

Description Vasopressin Injection is a clear and colorless liquid. It is odorless or has a slight, characteristic odor.

pH: 3.0 - 4.0

Purity Oxytocic principle—When tested by the following procedure, Vasopressin Injection contains not more than 0.6 oxytocin Units for each determined 10 vasopressin Units.

(i) Standard stock solution: Weigh accurately 0.02 to 0.05 g of Posterior Pituitary Reference Standard, place in a conical flask, and add exactly 0.5 mL of diluted acetic acid (100) (1 in 400) per 1.0 Unit. Insert a small funnel into the neck of the flask. Heat the mixture, with shaking gently in a water bath for 5 minutes. Cool the flask quickly to ordinary temperature, and filter: 1 mL of the filtrate is equivalent to 2.0 Unites. Place this filtrate in hard-glass ampules, seal and sterilize at 100°C for 30 minutes. Store in a cold place, and do not freeze. Use within 6 months from the date of preparation.

(ii) Standard solution: Dilute the standard stock solution with isotonic sodium chloride solution so that each mL of the solution contains 0.020 oxytocin Units.

(iii) Sample solution: Assume oxytocin Units as equivalent to 6/100 of the determined vasopressin Units. Dilute Vasopressin Injection with isotonic sodium chloride solution so that each mL of the resulting solution is expected to contain 0.020 oxytocin Unit.

(iv) Apparatus: Use the apparatus for the uterus contraction test, equipped with a thermostatic bath. Maintain a temperature of the bath at 37°C to 38°C with a variation of not more than 0.1°C during the course of the test. Use a 100-mL Magnus' chamber for suspending the uterus.

(v) Test animal: Use healthy, virgin and metestrus guinea pigs weighing between 175 g and 350 g. They have been bred under conditions where they have been completely isolated from the sight and smell of males since the time of weaning.

(vi) Procedure: Immerse the Magnus' chamber in the bath maintained at a constant temperature, add Locke-Ringer's solution to the chamber, and introduce oxygen into the solution at a moderate rate. Sacrifice a guinea pig by means of a blow on the head, immediately remove the uterus from the body, suspend it in the chamber, and connect one horn of the uterus to the lever with a thread. If necessary, weigh the lever provided that the mass is not changed throughout the assay. Start the assay after 15 to 30 minutes when the uterus is completely relaxed. Administer the same quantities, 0.1 to 0.5 mL, of the standard solution and the sample solution to the Magnus' chamber alternately twice with regular intervals of between 10 and 20 minutes to contract the uterus, finally administer the standard solution in a quantity which is 25% larger than the preceding doses, and measure the height of every contraction. The mean height of uterus contraction caused by the standard solution is equal to or higher than that caused by the sample solution. The height of contraction caused by the increased dose of the standard solution is distinctly higher than those caused by the preceding doses of the standard solution.

Assay (i) Test animals: Use healthy male rats weighing between 200 g and 300 g.

(ii) Standard stock solution: Use the standard stock solution obtained in Purity (i).

(iii) Standard solution: Dilute the standard stock solution with isotonic sodium chloride solution so that 0.2 mL of the obtained solution causes blood pressure increases of between 35 mmHg and 60 mmHg in test animals when injected according to (vi), and designate this solution as the high-dose standard solution (S_H). Then dilute this solution with isotonic sodium chloride solution 1.5 to 2.0 times by volume, and designate it as the low-dose standard solution (S_L).

(iv) Sample solution: Dilute an accurately measured volume of Vasopressin Injection with isotonic sodium chloride solution so that the obtained solution contains the same concentration in Units as the high-dose standard solution based on the labeled Units, and designate it as the high-dose sample solution (T_H). Then dilute this solution with isotonic sodium chloride solution 1.5 to 2.0 times by volume, and designate it as the low-dose sample solution (T_L). Make the concentration ratio of S_H to S_L equal to the ratio of T_H to T_L. When the sensitivity of an animal is changed, adjust the concentration of S_H and T_H before the next set of assay is started. However, keep the same ratio of S_H to S_L and T_H to T_L as in the primary set.

(v) Dose of injection: Although 0.2 mL of each solution is usually injected, the dose of injection can be determined based from preliminary tests or experiences. Inject the same volume throughout a set of tests.

(vi) Procedure: Inject subcutaneously 0.7 mL of a solution of ethyl carbamate (1 in 4) per 100 g of body mass to anesthetize the test animals and cannulate the trachea. Under artificial respiration (about 60 strokes per minute), remove a part of the second cervical vertebra, cut off the spinal cord and destroy the brain through the foramen magnum. Insert a cannula filled with isotonic sodium chloride solution into a femoral vein. Through this cannula, inject the solution prepared by dissolving 200 heparin Units of heparin sodium in 0.1 mL of isotonic sodium chloride solution, and then immediately inject 0.3 mL of isotonic sodium chloride solution. Insert a cannula into a carotid artery, and connect the cannula to a manometer for blood pressure measurement with a vinyl tube. The cannula and the vinyl tube have previously been filled with isotonic sodium chloride solution. Inject the standard and the sample solutions at regular intervals of 10 to 15 minutes into the femoral vein through the cannula followed by 0.3 mL of the isotonic solution when the blood pressure increases caused by each solution returns to the original level. Measure the height of blood pressure increases within 1 mmHg on the kymogram. Maintain a constant temperature between 20°C and 25°C during the assay. In advance, make four pairs from S_H, S_L, T_H, T_L as follows. Randomize the order of injection for pairs, but keep the order of injection within pairs as indicated.

Pair 1: S_H, T_L Pair 2: S_L, T_H Pair 3: T_H, S_L Pair 4: T_L, S_H

Carry out this assay using the same animals throughout a set of four pairs of observations. Perform this assay with two sets. If necessary, however, use the different animals for both sets of tests.

(vii) Calculation: Subtract increases of blood pressure caused by the low dose from those caused by the high dose in the Pair 1, 2, 3 and 4 of each set, and obtain the responses y_1, y_2, y_3 and y_4, respectively. Sum up y_1, for each set to obtain Y_1, and obtain Y_2, Y_3 and Y_4 in the same way.

$$\text{Units in each mL of Vasopressin Injection} = \text{antilog}\,M \times \left(\begin{array}{l}\text{Units in each ml of the}\\ \text{high-dose standard solution}\end{array}\right) \times \frac{b}{a}$$

$$M = \frac{IY_a}{Y_b}$$

$$I = \log\frac{S_H}{S_L} = \log\frac{T_H}{T_L}$$

$$Y_a = -Y_1 + Y_2 + Y_3 - Y_4$$

$$Y_b = Y_1 + Y_2 + Y_3 + Y_4$$

a: Volume (mL) of Vasopressin Injection sampled.

b: Total volume (mL) of the high-dose sample solution prepared by diluting with isotonic sodium chloride solution.

Compute L (P = 0.95) by the following equation, and confirm L to be 0.15 or less. If L exceeds 0.15, repeat the test, improving the conditions of the assay or increasing the number of sets until L reaches 0.15 or less.

$$L = 2\sqrt{(C-1)(CM^2 + I^2)}$$

$$C = \frac{Y_b^2}{Y_b^2 - 4fs^2t^2}$$

f: Number of sets

$$s^2 = \frac{\Sigma y^2 - \dfrac{Y}{f} - \dfrac{Y'}{4} + \dfrac{Y_b^2}{4f}}{n}$$

Σy^2: The sum of the squares of y_1, y_2, y_3 and y_4.

$Y = Y_1^2 + Y_2^2 + Y_3^2 + Y_4^2$

Y': The sum of the squares of the sum of y_1, y_2, y_3 and y_4 in each set.

$n = 3(f-1)$

t^2: Value shown in the table in the Assay under Insulin Injection against n for which s^2 is calculated.

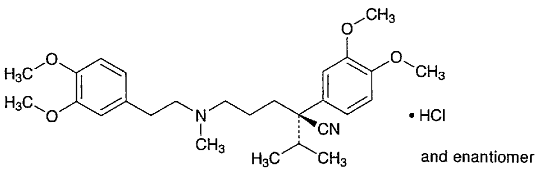
Containers and storage Containers—Hermetic containers. Storage—In a cold place, and avoid freezing.

Expiration date 36 months after preparation.

Verapamil Hydrochloride

Iproveratril Hydrochloride

塩酸ベラパミル



$C_{27}H_{38}N_2O_4.HCl$: 491.06

(*RS*)-5-[(3,4-Dimethoxyphenethyl)methylamino]-2-(3,4-dimethoxyphenyl)-2-(1-methylethyl)pentanenitrile monohydrochloride [*152-11-4*]

Verapamil Hydrochloride, when dried, contains not less than 98.5% of $C_{27}H_{38}N_2O_4.HCl$.

Description Verapamil Hydrochloride occurs as a white, crystalline powder. It is odorless.

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

Identification **(1)** To 2 mL of a solution of Verapamil Hydrochloride (1 in 50) add 5 drops of Reinecke salt TS: a light red precipitate is produced.

(2) Determine the absorption spectrum of a solution of Verapamil Hydrochloride in 0.01 mol/L hydrochloric acid TS (1 in 50,000) 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) Determine the infrared absorption spectrum of Verapamil 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 Verapamil Hydrochloride (1 in 50) responds to the Qualitative Tests for chloride.

Melting point 141 - 145°C

pH Dissolve 1.0 g of Verapamil Hydrochloride in 20 mL of freshly boiled and cooled water by warming, and cool: the pH of this solution is between 4.5 and 6.5.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Verapamil Hydrochloride in 20 mL of water by warming: the solution is clear and colorless.

(2) Heavy metals—Proceed with 1.0 g of Verapamil Hydrochloride 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).

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

(4) Related substances—Dissolve 0.50 g of Verapamil Hydrochloride in exactly 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 100 mL, and use this solution as the standard stock solution. Pipet 5 mL of the standard stock solution, add chloroform to make exactly 100 mL, and use this solution as the standard solution (1). Separately, pipet 5 mL of standard stock solution, add chloroform to make exactly 50 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the sample solution, the standard solutions (1) and (2) on two plates of silica gel for thin-layer chromatography. With the one plate, develop the plate with a mixture of cyclohexane and diethylamine (17:3) to a distance of about 15 cm, air-dry the plate, heat at 110°C for 1 hour, and cool. Examine immediately after spraying evenly iron (III) chloride-iodine TS on the plate: the three spots, having more intense color in the spots other than the principal spot and the original point from the sample solution, are not more intense than the spot from the standard solution (2) in color. The remaining spots from the sample solution are not more intense than the spot from the standard solution (1) in color. With another plate, develop the plate with a mixture of toluene, methanol, acetone and acetic acid (100) (14:4:1:1), and perform the test in the same manner.