on the dried basis.

Gas: Combustible gas—Acetylene gas
Supporting gas—Air
Lamp: Zinc hollow-cathode lamp
Wavelength: 213.9 nm

Loss on drying Not more than 10.0% (0.2 g, 105°C, 16 hours).

Residue on ignition Weigh accurately 0.02 to 0.04 g of Insulin in a tared platinum dish, add 2 drops of nitric acid, and heat the dish at first very gently and then strongly to incinerate. Place the dish in a muffle furnace, heat at 600°C for 15 minutes, cool in a desiccator (silica gel), and weigh: the mass of the residue is not more than 2.5%.

Nitrogen content Weigh accurately about 0.02 g of Insulin, dissolve in 10 mL of 0.1 mol/L hydrochloric acid TS, and perform the test as directed under the Nitrogen Determination: not less than 14.5% and not more than 16.5% of nitrogen (N: 14.01) is found, calculated on the dried basis.

Assay Proceed with Insulin as directed in the Assay under Insulin Injection with alterations in (v) Sample solution and (ix) Calculation as follows.

(v) Sample solution: Weigh accurately about 0.02 g of Insulin according to the labeled Units, dissolve with the diluent for insulin to make two different sample solutions, one to contain exactly 2.0 Units in each mL which is designated as the high-dose sample solution, T_H, and the other to contain exactly 1.0 Unit in each mL which is designated as the low-dose sample solution, T_L.

(ix) Calculation: Proceed as directed in the Assay (ix) Calculation under Insulin Injection, using the following equation,

$$\text{Units in each mg of Insulin} = \text{antilog } M \times \text{Units in each mL of the high-dosestandard solution} \times \frac{b}{a}$$

a: Mass (mg) of the sample,

instead of the following equation,

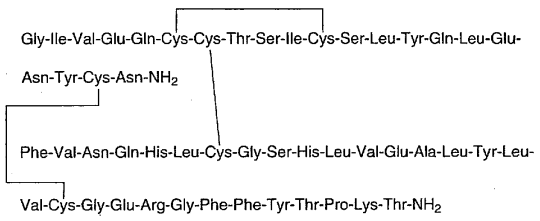
$$\text{Units in each mL of Insulin Injection} = \text{antilog } M \times \text{Units in each mL of the high-dose standard solution} \times \frac{b}{a}$$

a: Volume (mL) of the sample.

Contianers and storage Containers—Tight containers.
Storage—Not exceeding 8°C.

Insulin Human (Genetical Recombination)

ヒトインスリン(遺伝子組換え)



$C_{257}H_{383}N_{65}O_{77}S_6$: 5807.57
[11061-68-0]

Insulin Human (Genetical Recombination) is a human insulin prepared by genetical recombinant technology, and has an activity to reduce the blood sugar concentration. It contains not less than 27.5 Insulin Units per mg, calculated on the dried basis.

Description Insulin Human (Genetical Recombination) occurs as a white powder.

It is practically insoluble in water and in ethanol (95).

It dissolves in 0.01 mol/L hydrochloric acid TS and in sodium hydroxide TS with decomposition.

It is hygroscopic.

Identification Weigh accurately a suitable amount of Insulin Human (Genetical Recombination), and dissolve in 0.01 mol/L hydrochloric acid TS to make a solution so that each mL contains 2.0 mg. Transfer 500 μL of this solution into a clean test tube, add 2.0 mL of HEPES buffer solution, pH 7.5 and 400 μL of V8-protease TS, react at 25°C for 6 hours, then add 2.9 mL of ammonium sulfate buffer solution to stop the reaction, and use this solution as the sample solution. Separately, proceed with Human Insulin Reference Standard in the same manner as above, and use this solution as the standard solution. Perform the test with 50 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and compare the chromatograms obtained from these solutions: the peak appears just after the peak of the solvent and the succeeding three peaks with apparently higher peak height show the same retention time and similar peak height each other on both chromatograms.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 214 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

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

Mobile phase: Solution A—A mixture of water, ammonium sulfate buffer solution and acetonitrile (7:2:1). Solution B—A mixture of water, acetonitrile and ammonium sulfate buffer solution (2:2:1).

Change the mixing ratio of the solutions A and B linearly from 9:1 to 3:7 in 60 minutes after sample injection, further

change to 0:10 linearly in 5 minutes, and then flow the solution B only for 5 minutes.

Flow rate: 1.0 mL per minute.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the symmetry coefficients of the two larger peaks which appear next to the first peak just after the solvent peak are not more than 1.5, and the resolution between these peaks is not less than 3.4.

Purity **(1)** Related substances—Perform this procedure rapidly. Dissolve 7.5 mg of Insulin Human (Genetical Recombination) in 2 mL of 0.01 mol/L hydrochloric acid TS, and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under the Liquid Chromatography according to the following conditions, and determine the peak area of human insulin, A_1, the peak area of the desamide substance at the relative retention time of 1.3 to the human insulin, A_D, and the total area of the peaks other than the solvent peak, A_T: the amounts of the desamide substance and related substances other than the desamide substance are each not more than 2.0%. Previously, perform the test with 0.01 mol/L hydrochloric acid TS in the same manner to confirm the solvent peak.

$$\text{Amount (\%) of the desamide substance} = \frac{A_D}{A_T} \times 100$$

$$\text{Amount (\%) of related substances other than the desamide substance} = \frac{A_T - (A_1 + A_D)}{A_T} \times 100$$

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 214 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: Solution A—A mixture of phosphoric acid-sodium sulfate buffer solution, pH 2.3 and acetonitrile for liquid chromatography (41:9). Solution B—A mixture of phosphoric acid-sodium sulfate buffer solution, pH 2.3 and acetonitrile for liquid chromatography (1:1).

Flow a mixture of the solution A and the solution B (78:22) for 36 minutes before and after the sample injection, then change the mixing ratio to 33:67 linearly in 25 minutes, and maintain this ratio for 6 minutes. Then flow the first mixture (78:22) for the next 15 minutes. Adjust the mixing ratio of the first mixture so that the retention time of human insulin is about 25 minutes.

Flow rate: 1.0 mL per minute.

Time span of measurement: For about 75 minutes after the sample is injected.

System suitability—

Test for required detection: Confirm that the peak height of the desamide substance obtained from 20 μL of human insulin desamide substance-containing TS is between 30% and 70% of the full scale.

System performance: When the procedure is run with 20 μL of human insulin desamide substance-containing TS under the above operating conditions, human insulin and human insulin desamide substance are eluted in this order with the resolution between these peaks being not less than 2.0, and the symmetry coefficient of the peak of human insulin is not more than 1.8.

(2) High-molecular proteins—Dissolve 4 mg of Insulin Human (Genetical Recombination) in 1 mL of 0.01 mol/L hydrochloric acid TS. Perform the test with 100 μL of this solution as directed under the Liquid Chromatography according to the following conditions, and calculate each peak area: the total of areas of the peaks having smaller retention time than human insulin is not more than 1.0% of the total area of all peaks.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 276 nm).

Column: A stainless steel column 7.5 mm in inside diameter and 30 cm in length, packed with hydrophilic silica gel for liquid chromatography.

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of a solution of L-arginine (1 in 1000), acetonitrile and acetic acid (100) (13:4:3).

Flow rate: Adjust the flow rate so that the retention time of human insulin is about 20 minutes.

Time span of measurement: Until the peak of human insulin monomer has appeared.

System suitability—

Test for required detection: Confirm that the peak height of the dimer obtained from 100 μL of human insulin dimer containing TS is between 10% and 50% of the full scale.

System performance: When the procedure is run with 100 μL of human insulin dimer containing TS under the above operating conditions, polymer, dimer and monomer are eluted in this order, and the ratio, H_1/H_2, of the peak height of the dimer H_1 to the height of the bottom between the peaks of the dimer and the monomer H_2 is not less than 2.0.

(3) Product related impurities—Within the limits specified in each application dossier.

(4) Process related impurities—Within the limits specified in each application dossier.

Zinc content Weigh accurately about 0.05 g of Insulin Human (Genetical Recombination), and dissolve in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL. If necessary, dilute with 0.01 mol/L hydrochloric acid TS to make a solution so that each mL contains between 0.4 μg and 1.6 μg of zinc (Zn: 65.39), and use this solution as the sample solution. Separately, take exactly a suitable amount of Standard Zinc Solution for Atomic Absorption Spectrophotometry, dilute with 0.01 mol/L hydrochloric acid TS to make solutions containing 0.40 μg, 0.80 μg, 1.20 μg and 1.60 μg of zinc (Zn: 65.39) in each mL, respectively, and use these solutions as the standard solutions. Perform the test with the sample solution and the standard solutions as directed under the Atomic Absorption Spectrophotometry, and determine the amount of zinc in the sample solution by using a calibration curve obtained from the absorbances of the standard solutions: not more than 1.0%, calculated on the dried basis.

Gas: Combustible gas—Acetylene
Supporting gas—Air
Lamp: Zinc hollow cathode lamp
Wavelength: 213.9 nm

Loss on drying Not more than 10.0% (0.2 g, 105°C, 24 hours).

Bacterial endotoxins Less than 10 EU/mg.

Assay Perform this procedure quickly. Weigh accurately about 7.5 mg of Insulin Human (Genetical Recombination), dissolve in 0.01 mol/L hydrochloric acid TS to make exactly 5 mL, and use this solution as the sample solution. Separately, weigh accurately a suitable amount of Human Insulin Reference Standard, dissolve exactly in 0.01 mol/L hydrochloric acid TS to make a solution so that each mL contains about 40 Insulin Units, and use this solution as the standard solution. Perform the test with 20 μL 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 of human insulin, A_{TI} and A_{SI}, and the peak areas of the desamide substance at the relative retention time of 1.3 to the human insulin, A_{TD} and A_{SD}, respectively, of these solutions.

Amount (Insulin Unit/mg) of human insulin ($C_{257}H_{383}N_{65}O_{77}S_6$) $= \dfrac{W_S \times F}{C} \times \dfrac{A_{TI} + A_{TD}}{A_{SI} + A_{SD}} \times \dfrac{5}{W_T}$

F: Label unit (Insulin Unit/mg) of Human Insulin Reference Standard.

D: Volume (mL) of 0.01 mol/L hydrochloric acid TS used to dissolve the reference standard.

W_T: Amount (mg) of the sample calculated on the dried basis.

W_S: Amount (mg) of Human Insulin Reference Standard.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 214 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 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: A mixture of phosphoric acid-sodium sulfate buffer solution, pH 2.3 and acetonitrile for liquid chromatography (3:1). Adjust the mixing ratio of the component of the mobile phase so that the retention time of human insulin is between 10 minutes and 17 minutes.

Flow rate: 1.0 mL per minute.

System suitability—

System performance: When the procedure is run with 20 μL of human insulin desamide substance-containing TS under the above operating conditions, human insulin and human insulin desamide substance are eluted in this order with the resolution between these peaks being not less than 2.0, and the symmetry coefficient of the peak of human insulin is not more than 1.8.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of human insulin is not more than 1.6%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and at −20°C or below.

Insulin Injection

インスリン注射液

Insulin Injection is an aqueous solution for injection. It contains not less than 95% and not more than 105% of the labeled Insulin Units.

Method of preparation Suspend Insulin in Water for Injection, dissolve by adding Hydrochloric Acid, and prepare as directed under Injections. It contains 0.10 to 0.25 g of Phenol or Cresol and 1.4 to 1.8 g of Concentrated Glycerin for each 100 mL of Insulin Injection. It should not contain sodium chloride.

Description Insulin Injection is a clear, colorless or pale yellow liquid.

Identification Adjust Insulin Injection to pH between 5.1 and 5.3 with a solution of sodium hydroxide (1 in 100): a precipitate is produced. Adjust the solution to a pH between 2.5 and 3.5 with dilute hydrochloric acid: the precipitate dissolves.

pH 2.5 – 3.5

Residue on ignition Measure exactly a volume of Insulin Injection, equivalent to 500 to 1000 Units according to the labeled Units, in a tared platinum dish, and evaporate slowly by heating on a water bath to dryness. Add 2 drops of nitric acid to the residue, and heat at first very gently, then strongly to incinerate. Place in a muffle furnace, and heat at 600°C for 15 minutes, cool in a desiccator (silica gel), and weigh: the mass of the residue is not more than 1.0 mg for each labeled 1000 Units.

Nitrogen content Perform the test as directed under the Nitrogen Determination: not less than 0.50 mg and not more than 0.64 mg of nitrogen (N: 14.01) is found for each labeled 100 Units.

Assay (i) Animals: Select healthy rabbits each weighing not less than 1.8 kg. Keep the rabbits in the laboratory not less than 1 week before use in the assay by feeding them with an appropriate uniform diet and water.

(ii) Diluent for insulin: Dissolve 1.0 to 2.5 g of phenol or *m*-cresol in 500 mL of 0.01 mol/L hydrochloric acid VS, and add 14 to 18 g of glycerin and 0.01 mol/L hydrochloric acid VS to make 1000 mL.

(iii) Standard stock solution: Weigh accurately about 0.02 g of Insulin Reference Standard, and dissolve it in the diluent for insulin to make a standard stock solution containing exactly 20.0 Units in each mL. Preserve this solution between 1°C and 15°C, and use within 6 months.

(iv) Standard solution: Dilute two portions of the standard stock solution to make two standard solutions with the diluent for insulin, one to contain exactly 2.0 Units in each mL which is designated as the high-dose standard solution S_H, and the other to contain exactly 1.0 Unit in each mL which is designated as the low-dose standard solution S_L.

(v) Sample solution: According to the labeled Units, dilute two portions of Insulin Injection to make two sample solutions with the diluent for insulin, one to contain exactly 2.0 Units in each mL which is designated as the high-dose sample solution T_H, and the other to contain exactly 1.0