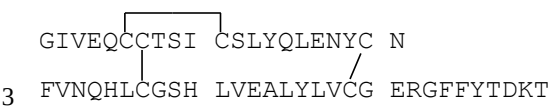
Insulin Aspart (Genetical Recombination)

インスリン　アスパルト（遺伝子組換え）



$C_{256}H_{381}N_{65}O_{79}S_6$: 5825.54

[*116094-23-6*]

Insulin Aspart (Genetical Recombination) is an analogue of human insulin (genetical recombination), being substituted proline residue with aspartic acid residue at 28th of B chain. It is a peptide composed of A chain consisting of 21 amino acid residues and B chain consisting of 30 amino acid residues.

It contains not less than 92.6% and not more than 109.5% of insulin aspart (genetical recombination) ($C_{256}H_{381}N_{65}O_{79}S_6$), calculated on the dried and residue on ignition-free basis.

0.0350 mg of Insulin Aspart (Genetical Recombination) is equivalent to 1 Insulin Unit.

Description Insulin Aspart (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.

It is hygroscopic.

Identification Weigh a suitable amount of Insulin Aspart (Genetical Recombination) and Insulin Aspart RS, and dissolve each in 0.01 mol/L hydrochloric acid TS so that each mL contains 2.0 mg of insulin aspart. Transfer 25 μL of these solutions into clean test tubes, add 100 μL of HEPES buffer solution (pH 7.5) and 20 μL of V8-protease TS, and allow to react at 25℃ for 6 hours. Then add 145 μL of ammonium sulfate buffer solution to stop the reaction, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and compare the peak eluted just after the peak of the solvent and the succeeding three peaks with apparently higher peak height in the chromatograms obtained from these solutions: the similar peaks are observed at the same retention times.

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 (not exceeding 5 μm in particle diameter).

Column temperature: A constant temperature of about 40℃.

Mobile phase A: A mixture of water, ammonium sulfate buffer solution and acetonitrile for liquid chromatography (7:2:1).

Mobile phase B: A mixture of water, acetonitrile for liquid chromatography and ammonium sulfate buffer solution (2:2:1).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 － 60	90 → 30	10 → 70
60 － 65	30 → 0	70 → 100
65 － 70	0	100

Flow rate: 1 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 peaks of digestion fragment I, II, III and IV are observed. The symmetry factors of the peaks of digestion fragment II and III are not more than 1.5, and the resolution between these peaks is not less than 8.0.

Purity **(1)** Related substances－Perform the test with 10 μL of the sample solution obtained in the Assay as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amounts of them by the area percentage method: the amount of the peak of B28isoAsp insulin aspart, having the relative retention time of about 0.9 to insulin aspart, is not more than 0.3%, the total amount of the peak of A21Asp insulin aspart and B3Asp insulin aspart, having the relative retention time of about 1.3 to insulin aspart, and the peak of B3isoAsp insulin aspart, having the relative retention time of about 1.5 to insulin aspart, is not more than 1.0%, and the total amount of the peaks other than the peaks mentioned above is not more than 0.5%.

Operating conditions －

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

Time span of measurement: From 4 minutes to 50 minutes after injection of the sample solution.

System suitability －

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

Test for required detectability: Allow Insulin Aspart RS to stand at an ordinary temperature for 5 days, dissolve in 0.01 mol/L hydrochrolic acid TS so that each mL contains 4.0 mg of insulin aspart. Allow this solution to stand at an ordinary temperature for 1 to 3 days, and use this solution as the solution for system suitability test. The solution for system suitability test contains not less than 0.1% and not more than 2.2% of B28isoAsp insulin aspart, and not less than 1% of B3Asp insulin aspart and A21Asp insulin aspart. Store the solution for system suitability test at a temperature between 2℃ and 8℃, and use within 72 hours. Pipet 5 mL of the solution for system suitability test, add 0.01 mol/L hydrochloric acid TS to make exactly 10 mL. Confirm that the

area percentage of the peak of B28isoAsp insulin aspart obtained with 10 μL of this solution is equivalent to 80 to 120% of that obtained with 10 μL of the solution for system suitability test.

(2) High-molecular proteins－Store the sample solution at a temperature between 2℃ and 8℃, and use within 48 hours after preparation. Dissolve 4 mg of Insulin Aspart (Genetical Recombination) in 1 mL of 0.01 mol/L hydrochloric acid TS, and use this solution as the sample solution. Perform the test with 100 μL of the sample solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, determine each peak area by the automatic integration method, and calculate the amounts of them by the area percentage method: the total amount of the peaks other than insulin aspart monomer is not more than 0.3%.

Operating conditions －

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

Column: A stainless steel column 7.8 mm in inside diameter and 30 cm in length, packed with hydrophilic silica gel for liquid chromatography (5 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 20℃.

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

Flow rate: 0.5 mL per minute.

Time span of measurement: Until the elution of insulin aspart monomer is completed.

System suitability －

Test for required detectability: Allow Insulin Aspart (Genetic Recombination) to stand at an ordinary temperature for about 10 days, dissolve in 0.01 mol/L hydrochloride TS so that the solution contains about 0.4% of high-molecular proteins and each mL contains about 4 mg of insulin aspart, and use this solution as the solution for system suitability test. Store the solution for system suitability test at a temperature between 2℃ and 8℃, and use within 7 days. Pipet 5 mL of the solution for system suitability test, add 0.01 mol/L hydrochloric acid TS to make exactly 10 mL. Confirm that the area percentage of the peak of insulin aspart dimer obtained with 100 μL of this solution is equivalent to 80 to 120% of that obtained with 100 μL of the solution for system suitability test.

System performance: When the procedure is run with 100 μL of the solution for system suitability test under the above operating conditions, insulin aspart polymer (retention time: 13 to 17 minutes), insulin aspart dimer (retention time: about 17.5 minutes) and insulin aspart monomer (retention time: 18 to 20 minutes) 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.

System repeatability: When the test is repeated 6 times with 100 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of insulin aspart monomer is not more than 2.0%.

(3) Host cell proteins－Being specified separately when the drug is granted approval based on the Law.

(4) DNA － Being specified separately when the drug is granted approval based on the Law.

Loss on drying *<2.41>* Not more than 10.0% (0.2 g, 105℃, 24 hours).

Residue on ignition *<2.44>* Not more than 6.0% (0.2 g).

Assay Store the sample solution and the standard solution at between 2℃ and 8℃, use the sample solution within 24 hours after preparation, and use the standard solution within 48 hours after preparation. Weigh accurately a suitable amout of Insulin Aspart (Genetical Recombination) and Insulin Aspart RS (separately determine the loss on drying *<2.41>* and the residue on ignition *<2.44>* under the same conditions as Insulin Aspart (Genetic Recombination)), dissolve each in 0.01 mol/L hydrochloric acid TS so that each mL contains 4.0 mg of insulin aspart, and use these solutions as the sample solution and the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and determine the total areas, A_T and A_S, of the peak of B28isoAsp insulin aspart (relative retention time to insulin aspart: about 0.9), the peak of insulin aspart (retention time: 20 to 24 minutes), the peak of A21Asp insulin aspart and B3Asp insulin aspart (usually eluted together having the relative retention time of about 1.3 to insulin aspart) and the peak of B3iso-Asp insulin aspart (relative retention time to insulin aspart: about 1.5) in each solution.

Amount (mg) of insulin aspart ($C_{256}H_{381}N_{65}O_{79}S_6$)
$= M_S \times A_T / A_S$

M_S: Amount (mg) Insulin Aspart RS in 1 mL of the standard solution, calculated on the dried and residue on ignition-free basis

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 (not exceeding 5 μm in particle diameter).

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

Mobile phase A: Dissolve 142.0 g of anhydrous sodium sulfate in water, add 13.5 mL of phosphoric acid, and add water to make 5 L. Adjust to pH 3.6 with sodium hydroxide TS. To 4500 mL of this solution add 500 mL of acetonitrile for liquid chromatography.

Mobile phase B: A mixture of water and acetonitrile for liquid chromatography (1:1).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 － 35	58	42
35 － 40	58 → 20	42 → 80
40 － 45	20	80
45 － 46	20 → 58	80 → 42
46 － 60	58	42

Flow rate: 1 mL per minute.

System suitability－

System performance: Allow Insulin Aspart RS to stand at an ordinary temperature for 5 days, dissolve in 0.01 mol/L hydrochrolic acid TS so that each mL contains 4.0 mg of insulin aspart. Allow this solution to stand at an ordinary temperature for 1 to 3 days, and use this solution as the solution for system suitability test. The solution for system suitability test contains not less than 0.1% and not more than 2.2% of B28isoAsp insulin aspart, and not less than 1% of B3Asp insulin aspart and A21Asp insulin aspart. Store the solution for system suitability test at a temperature between 2℃ and 8℃, and use within 72 hours. When the procedure is run with 10 μL of the solution for system suitability test under the above operating conditions, B28isoAsp insulin aspart, insulin aspart, A21Asp insulin aspart and B3Asp insulin aspart, and B3isoAsp insulin aspart are eluted in this order with the resolution between the peak of insulin aspart and the peak of A21Asp insulin aspart and B3Asp insulin aspart being not less than 2.0.

System repeatability: When the test is repeated 5 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of A_S is not more than 1.5%.

Containers and storage Containers－Tight containers.

Storage－Light-resistant, not exceeding -18℃.

Add the following to 9.01 Reference Standards (1):

Insulin Aspart RS