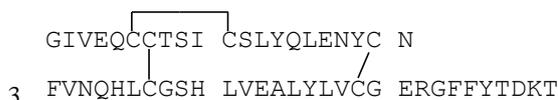


## 1 Insulin Aspart (Genetical Recombination)

2 インスリン アスパルト (遺伝子組換え)



4 C<sub>256</sub>H<sub>381</sub>N<sub>65</sub>O<sub>79</sub>S<sub>6</sub>: 5825.54

5 [116094-23-6]

6

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

13 It contains not less than 92.6% and not more than 109.5%  
14 of insulin aspart (genetical recombination)  
15 (C<sub>256</sub>H<sub>381</sub>N<sub>65</sub>O<sub>79</sub>S<sub>6</sub>), calculated on the dried and residue on ig-  
16 nition-free basis.

17 0.0350 mg of Insulin Aspart (Genetical Recombination) is  
18 equivalent to 1 Insulin Unit.

19 **Description** Insulin Aspart (Genetical Recombination) occurs  
20 as a white powder.

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

22 It dissolves in 0.01 mol/L hydrochloric acid TS.

23 It is hygroscopic.

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

39 **Operating conditions** —

40 **Detector:** An ultraviolet absorption photometer (wavelength:  
41 214 nm).

42 **Column:** A stainless steel column 4.6 mm in inside diameter  
43 and 10 cm in length, packed with octadecylsilanized silica gel for  
44 liquid chromatography (not exceeding 5  $\mu$ m in particle diameter).

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

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

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

50 **Flowing of mobile phase:** Control the gradient by mixing the  
51 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

53

54 **Flow rate:** 1 mL per minute.

55 **System suitability** —

56 **System performance:** When the procedure is run with 50  $\mu$ L of  
57 the standard solution under the above operating conditions, the  
58 peaks of digestion fragment I, II, III and IV are observed. The  
59 symmetry factors of the peaks of digestion fragment II and III are  
60 not more than 1.5, and the resolution between these peaks is not  
61 less than 8.0.

62 **Purity (1)** Related substances—Perform the test with 10  $\mu$ L  
63 of the sample solution obtained in the Assay as directed under Liq-  
64 uid Chromatography <2.01> according to the following conditions.  
65 Determine each peak area by the automatic integration method,  
66 and calculate the amounts of them by the area percentage method:  
67 the amount of the peak of B28isoAsp insulin aspart, having the  
68 relative retention time of about 0.9 to insulin aspart, is not more  
69 than 0.3%, the total amount of the peak of A21Asp insulin aspart  
70 and B3Asp insulin aspart, having the relative retention time of  
71 about 1.3 to insulin aspart, and the peak of B3isoAsp insulin aspart,  
72 having the relative retention time of about 1.5 to insulin aspart, is  
73 not more than 1.0%, and the total amount of the peaks other than  
74 the peaks mentioned above is not more than 0.5%.

75 **Operating conditions** —

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

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

81 **System suitability** —

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

84 **Test for required detectability:** Allow Insulin Aspart RS to stand  
85 at an ordinary temperature for 5 days, dissolve in 0.01 mol/L  
86 hydrochloric acid TS so that each mL contains 4.0 mg of insulin  
87 aspart. Allow this solution to stand at an ordinary temperature for  
88 1 to 3 days, and use this solution as the solution for system  
89 suitability test. The solution for system suitability test contains not  
90 less than 0.1% and not more than 2.2% of B28isoAsp insulin  
91 aspart, and not less than 1% of B3Asp insulin aspart and A21Asp  
92 insulin aspart. Store the solution for system suitability test at a  
93 temperature between 2°C and 8°C, and use within 72 hours. Pipet  
94 5 mL of the solution for system suitability test, add 0.01 mol/L  
95 hydrochloric acid TS to make exactly 10 mL. Confirm that the

96 area percentage of the peak of B28isoAsp insulin aspart obtained 149  
 97 with 10  $\mu\text{L}$  of this solution is equivalent to 80 to 120% of that 150  
 98 obtained with 10  $\mu\text{L}$  of the solution for system suitability test. 151

99 (2) High-molecular proteins—Store the sample solution at a 152  
 100 temperature between 2°C and 8°C, and use within 48 hours after 153  
 101 preparation. Dissolve 4 mg of Insulin Aspart (Genetical Recombination) 154  
 102 in 1 mL of 0.01 mol/L hydrochloric acid TS, and use this 155  
 103 solution as the sample solution. Perform the test with 100  $\mu\text{L}$  of 156  
 104 the sample solution as directed under Liquid Chromatography 157  
 105 <2.01> according to the following conditions, determine each peak 158  
 106 area by the automatic integration method, and calculate the 159  
 107 amounts of them by the area percentage method: the total amount 160  
 108 of the peaks other than insulin aspart monomer is not more than 161  
 109 0.3%. 162

110 *Operating conditions* —

111 Detector: An ultraviolet absorption photometer (wavelength: 163  
 112 276 nm). 164

113 Column: A stainless steel column 7.8 mm in inside diameter 165  
 114 and 30 cm in length, packed with hydrophilic silica gel for liquid 166  
 115 chromatography (5 to 10  $\mu\text{m}$  in particle diameter). 167

116 Column temperature: A constant temperature of about 20°C. 168

117 Mobile phase: A mixture of a solution of L-arginine (1 in 1000), 169  
 118 acetonitrile for liquid chromatography and acetic acid (100 170  
 119 (13:4:3)). 171

120 Flow rate: 0.5 mL per minute. 172

121 Time span of measurement: Until the elution of insulin aspart 173  
 122 monomer is completed. 174

123 *System suitability* —

124 Test for required detectability: Allow Insulin Aspart (Genetic 175  
 125 Recombination) to stand at an ordinary temperature for about 10 176  
 126 days, dissolve in 0.01 mol/L hydrochloride TS so that the solution 177  
 127 contains about 0.4% of high-molecular proteins and each mL 178  
 128 contains about 4 mg of insulin aspart, and use this solution as the 179  
 129 solution for system suitability test. Store the solution for system 180  
 130 suitability test at a temperature between 2°C and 8°C, and use 181  
 131 within 7 days. Pipet 5 mL of the solution for system suitability test, 182  
 132 add 0.01 mol/L hydrochloric acid TS to make exactly 10 mL. 183  
 133 Confirm that the area percentage of the peak of insulin aspart 184  
 134 dimer obtained with 100  $\mu\text{L}$  of this solution is equivalent to 80 to 185  
 135 120% of that obtained with 100  $\mu\text{L}$  of the solution for system 186  
 136 suitability test. 187

137 System performance: When the procedure is run with 100  $\mu\text{L}$  188  
 138 of the solution for system suitability test under the above operating 189  
 139 conditions, insulin aspart polymer (retention time: 13 to 17 190  
 140 minutes), insulin aspart dimer (retention time: about 17.5 minutes) 191  
 141 and insulin aspart monomer (retention time: 18 to 20 minutes) are 192  
 142 eluted in this order, and the ratio,  $H_1/H_2$ , of the peak height of the 193  
 143 dimer  $H_1$  to the height of the bottom between the peaks of the 194  
 144 dimer and the monomer  $H_2$  is not less than 2.0. 195

145 System repeatability: When the test is repeated 6 times with 100  
 146  $\mu\text{L}$  of the solution for system suitability test under the above  
 147 operating conditions, the relative standard deviation of the peak  
 148 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°C, 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 be-  
 tween 2°C and 8°C, use the sample solution within 24 hours after  
 preparation, and use the standard solution within 48 hours after  
 preparation. Weigh accurately a suitable amount of Insulin Aspart  
 (Genetical Recombination) and Insulin Aspart RS (separately de-  
 termine the loss on drying <2.41> and the residue on ignition  
 <2.44> under the same conditions as Insulin Aspart (Genetic Re-  
 combination)), 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  $\mu\text{L}$  each of the sample solution and standard solu-  
 tion 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.

$$\begin{aligned} & \text{Amount (mg) of insulin aspart (C}_{256}\text{H}_{381}\text{N}_{65}\text{O}_{79}\text{S}_6) \\ & = M_S \times A_T / A_S \end{aligned}$$

$M_S$ : 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  $\mu\text{m}$  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.

196

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

197

198 Flow rate: 1 mL per minute.

199 *System suitability* –

200 System performance: Allow Insulin Aspart RS to stand at an  
 201 ordinary temperature for 5 days, dissolve in 0.01 mol/L  
 202 hydrochloric acid TS so that each mL contains 4.0 mg of insulin  
 203 aspart. Allow this solution to stand at an ordinary temperature for  
 204 1 to 3 days, and use this solution as the solution for system  
 205 suitability test. The solution for system suitability test contains not  
 206 less than 0.1% and not more than 2.2% of B28isoAsp insulin  
 207 aspart, and not less than 1% of B3Asp insulin aspart and A21Asp  
 208 insulin aspart. Store the solution for system suitability test at a  
 209 temperature between 2°C and 8°C, and use within 72 hours. When  
 210 the procedure is run with 10 µL of the solution for system  
 211 suitability test under the above operating conditions, B28isoAsp  
 212 insulin aspart, insulin aspart, A21Asp insulin aspart and B3Asp  
 213 insulin aspart, and B3isoAsp insulin aspart are eluted in this order  
 214 with the resolution between the peak of insulin aspart and the peak  
 215 of A21Asp insulin aspart and B3Asp insulin aspart being not less  
 216 than 2.0.

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

220 **Containers and storage** Containers—Tight containers.

221 Storage—Light-resistant, not exceeding -18°C.

222 **Add the following to 9.01 Reference Stand-**  
 223 **ards (1):**

224 **Insulin Aspart RS**