

1 Insulin Aspart (Genetical Recombination)

2 インスリン アスパルト (遺伝子組換え)



4 $C_{256}H_{381}N_{65}O_{79}S_6$: 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 ($C_{256}H_{381}N_{65}O_{79}S_6$), 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 μ L of these solutions into clean
28 test tubes, add 100 μ L of HEPES buffer solution (pH 7.5) and 20
29 μ L of V8-protease TS, and allow to react at 25°C for 6 hours.
30 Then add 145 μ 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 μ 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 μ 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 μ 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 μ 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 μL of this solution is equivalent to 80 to 120% of that 150
 98 obtained with 10 μL 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 μL 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 μm 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 μL of this solution is equivalent to 80 to 185
 135 120% of that obtained with 100 μL of the solution for system 186
 136 suitability test. 187

137 System performance: When the procedure is run with 100 μL 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 μL 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 μL 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 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.

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 Standards (1):**
 223 **ards (1):**

224 **Insulin Aspart RS**