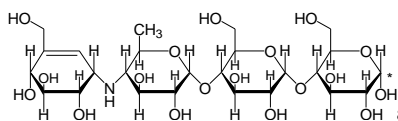


1 **Acarbose**

2 アカルボース



3 and epimer at C*

4 $C_{25}H_{43}NO_{18}$: 645.60

5 *O*-4,6-Dideoxy-4-[[[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclo-
6 hex-2-en-1-yl]amino]- α -D-glucopyranosyl-(1 \rightarrow 4)-*O*- α -D-glucopyranosyl-
7 (1 \rightarrow 4)-D-glucopyranose
8 [56180-94-0]

9

10 Acarbose is an oligosaccharide having α -amylase and
11 β -amylase inhibiting activity produced by the growth of
12 *Actinoplanes utahensis*.

13 Acarbose contains not less than 95.0% and not more than
14 101.0% of acarbose ($C_{25}H_{43}NO_{18}$), calculated on the
15 anhydrous basis.

16 **Description** Acarbose occurs as a white to light yellow powder.

17 It is very soluble in water, soluble in methanol, and slightly sol-
18 uble in ethanol (99.5).

19 It is hygroscopic.

20 **Identification** Determine the infrared absorption spectrum of
21 Acarbose as directed in the potassium bromide disk method under
22 Infrared Spectrophotometry <2.25>, and compare the spectrum
23 with the Reference Spectrum or the spectrum of Acarbose RS:
24 both spectra exhibit similar intensities of absorption at the same
25 wave numbers.

26 **Optical rotation** <2.49> $[\alpha]_D^{20}$: +171 – +181° (0.5 g calculated
27 on the anhydrous basis, water, 50 mL, 100 mm).

28 **pH** <2.54> Dissolve 1.0 g of Acarbose in freshly boiled and
29 cooled water to make 20 mL: the pH of this solution is between
30 5.5 and 7.5.

31 **Purity (1)** Chloride <1.03>—Perform the test with 0.10 g of
32 Acarbose. Prepare the control solution with 1.40 mL of 0.01 mol/L
33 hydrochloric acid VS (not more than 0.496%).

34 **(2)** Heavy metals <1.07>—Proceed with 2.0 g of Acarbose
35 according to Method 4, and perform the test. Prepare the control
36 solution with 2.0 mL of Standard Lead Solution (not more than 10
37 ppm).

38 **(3)** Related substances—Use the sample solution obtained in
39 the Assay as the sample solution. Pipet 1 mL of the sample solu-
40 tion, add water to make exactly 100 mL, and use this solution as
41 the standard solution. Perform the test with exactly 10 μ L each of
42 the sample solution and standard solution as directed under Liquid
43 Chromatography <2.01> according to the following conditions,
44 and determine each peak area by the automatic integration
45 method: the peak area of the related substance D having the rela-

46 tive retention time of about 0.5 to acarbose from the sample solu-
47 tion is not larger than the peak area of acarbose from the standard
48 solution, the peak areas of the related substance B having the rela-
49 tive retention time of about 0.8 to acarbose, the related substance
50 A having the relative retention time of about 0.9, the related sub-
51 stance C having the relative retention time of about 1.2, the related
52 substance E having the relative retention time of about 1.7, the
53 related substance F having the relative retention time of about 1.9
54 and the related substance G having the relative retention time of
55 about 2.2 from the sample solution are not larger than 1/2, 3/5, 1.5,
56 1/5, 3/10 and 3/10 times the peak area of acarbose from the stand-
57 ard solution, respectively, and the area of the peak other than acar-
58 bose and the peaks mentioned above from the sample solution is
59 not larger than 1/5 times the peak area of acarbose from the stand-
60 ard solution. Furthermore, the total area of the peaks of the related
61 substance D, B, A, C, E, F and G from the sample solution is not
62 larger than 3 times the peak area of acarbose from the standard
63 solution. For the peak areas of the related substance D, B, E, F and
64 G, multiply their relative response factors, 0.75, 0.63, 1.25, 1.25
65 and 1.25, respectively.

66 **Operating conditions** —

67 Detector, column, column temperature, mobile phase and flow
68 rate: Proceed as directed in the operating conditions in the Assay.

69 Time span of measurement: About 2.5 times as long as the
70 retention time of acarbose.

71 **System suitability** —

72 System performance: Proceed as directed in the system
73 suitability in the Assay.

74 Test for required detectability: To 5 mL of the standard solution
75 add water to make 100 mL. When the procedure is run with this
76 solution under the above operating conditions, the SN ratio of the
77 peak of acarbose is more than 10.

78 System repeatability: Pipet 1 mL of the standard solution
79 obtained in the Assay, and add water to make exactly 100 mL.
80 When the test is repeated 6 times with 10 μ L of this solution under
81 the above operating conditions, the relative standard deviation of
82 the peak area of acarbose is not more than 2.0%.

83 **Water** <2.48> Not more than 4.0% (0.3 g, volumetric titration,
84 direct titration).

85 **Residue on ignition** <2.44> Not more than 0.2% (1 g).

86 **Assay** Weigh accurately about 0.1 g of Acarbose, dissolve in
87 water to make exactly 5 mL, and use this solution as the sample
88 solution. Separately, weigh about 0.1 g of Acarbose RS (sepa-
89 rately determine the water <2.48> in the same manner as Acarbose),
90 add exactly 5 mL of water, and use this solution as the standard
91 solution. Perform the test with exactly 10 μ L each of the sample
92 solution and standard solution as directed under Liquid Chroma-
93 tography <2.01> according to the following conditions, and deter-
94 mine the peak areas, A_T and A_S , of acarbose in each solution.

95 Amount (mg) of acarbose ($C_{25}H_{43}NO_{18}$) = $M_S \times A_T / A_S$

96 M_S : Amount (mg) of Acarbose RS taken, calculated on the an-
97 hydrous basis

98 **Operating conditions** —

99 Detector: An ultraviolet absorption photometer (wavelength:
100 210 nm).

101 Column: A stainless steel column 4 mm in inside diameter and
102 25 cm in length, packed with aminopropylsilanized silica gel for
103 liquid chromatography (5 μ m in particle diameter).

104 Column temperature: A constant temperature of about 35°C.

105 Mobile phase: Dissolve 0.60 g of potassium
106 dihydrogenphosphate and 0.35 g of disodium hydrogenphosphate
107 dihydrate in 1000 mL of water. To 250 mL of this solution add
108 750 mL of acetonitrile for liquid chromatography.

109 Flow rate: 2.0 mL per minute.

110 **System suitability** —

111 System performance: Dissolve Acarbose RS for System
112 Suitability in water to make a solution so that each mL contains
113 20 mg/mL. When the procedure is run with 10 μ L of this solution
114 under the above operating conditions, the peaks of the related
115 substance D having the relative retention time of about 0.5 to
116 acarbose, the related substance B having the relative retention time
117 of about 0.8, the related substance A having the relative retention
118 time of about 0.9, the related substance C having the relative
119 retention time of about 1.2, the related substance E having the
120 relative retention time of about 1.7, the related substance F having
121 the relative retention time of about 1.9 and the related substance
122 G having the relative retention time of about 2.2 are observed, and
123 the peak-valley ratio between the peaks of the related substance A
124 and acarbose is not less than 1.2.

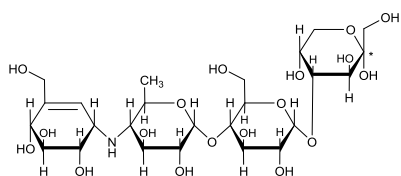
125 System repeatability: When the test is repeated 6 times with 10
126 μ L of the standard solution under the above operating conditions,
127 the relative standard deviation of the peak area of acarbose is not
128 more than 1.0%.

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

130 **Others**

131 Related substance A:

132 *O*-4,6-Dideoxy-4-[[*(1S,4R,5S,6S)*-4,5,6-trihydroxy-3-
133 (hydroxymethyl)cyclohex-2-en-1-yl]amino]- α -D-
134 glucopyranosyl-(1 \rightarrow 4)-*O*- α -D-glucopyranosyl-(1 \rightarrow 4)-*D*-*arab*-
135 *ino*-hex-2-ulopyranose



136 and epimer at C*

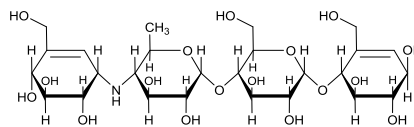
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139 Related substance B:

140 (*1R,4R,5S,6R*)-4,5,6-Trihydroxy-2-(hydroxymethyl)cyclohex-2-
141 en-1-yl 4-*O*-(4,6-dideoxy-4-[[*(1S,4R,5S,6S)*-4,5,6-

142 trihydroxy-3-(hydroxymethyl)cyclohex-2-en-1-yl]amino)-
143 α -D-glucopyranosyl)- α -D-glucopyranoside



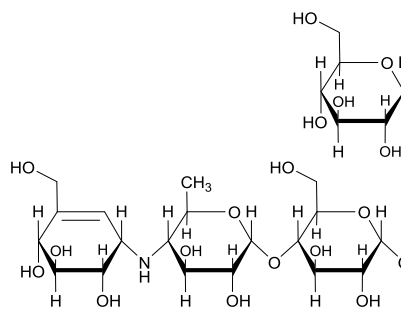
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147 Related substance C:

148 α -D-Glucopyranosyl 4-*O*-(4,6-dideoxy-4-[[*(1S,4R,5S,6S)*-4,5,6-
149 trihydroxy-3-(hydroxymethyl)cyclohex-2-en-1-yl]amino)- α -D-
150 glucopyranosyl)- α -D-glucopyranoside



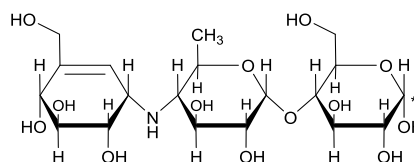
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154 Related substance D:

155 4-*O*-(4,6-Dideoxy-4-[[*(1S,4R,5S,6S)*-4,5,6-trihydroxy-3-
156 (hydroxymethyl)cyclohex-2-en-1-yl]amino)- α -D-
157 glucopyranosyl)-D-glucopyranose



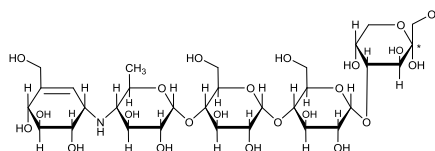
158 and epimer at C*

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161 Related substance E:

162 *O*-4,6-Dideoxy-4-[[*(1S,4R,5S,6S)*-4,5,6-trihydroxy-3-
163 (hydroxymethyl)cyclohex-2-en-1-yl]amino)- α -D-
164 glucopyranosyl-(1 \rightarrow 4)-*O*- α -D-glucopyranosyl-(1 \rightarrow 4)-*O*- α -D-
165 glucopyranosyl-(1 \rightarrow 4)-D-*arabino*-hex-2-ulopyranose
166 (4-*O*- α -Acarbosyl-D-fructopyranose)



167

and epimer at C*

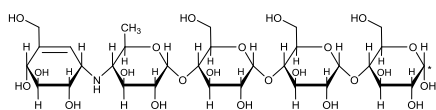
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170 Related substance F:

171 *O*-4,6-Dideoxy-4-[[*(1S,4R,5S,6S)*-4,5,6-trihydroxy-3-
172 (hydroxymethyl)cyclohex-2-en-1-yl]amino)- α -D-

- 173 glucopyranosyl-(1→4)-*O*- α -D-glucopyranosyl-(1→4)-*O*- α -D-
 174 glucopyranosyl-(1→4)-D-glucopyranose
 175 (4-*O*- α -Acarbosyl-D-glucopyranose)



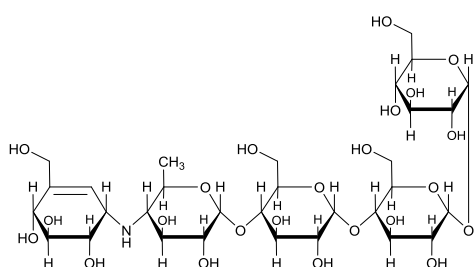
- 176 and epimer at C*

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179 Related substance G:

- 180 α -D-Glucopyranosyl *O*-4,6-dideoxy-4-[(1*S*,4*R*,5*S*,6*S*)-4,5,6-tri-
 181 hydroxy-3-(hydroxymethyl)cyclohex-2-en-1-yl]amino}-
 182 α -D-glucopyranosyl-(1→4)-*O*- α -D-glucopyranosyl-(1→4)-
 183 *O*- α -D-glucopyranoside (α -D-Glucopyranosyl α -acarbose)



184

- 185 **Add the following to 9.01 Reference Standards**
 186 **(1) :**

187 **Acarbose RS**

188 **Acarbose RS for System Suitability**

189

190 **Add the following to 9.41 Reagents, Test**

191 **Solutions:**

192 **Disodium hydrogenphosphate dihydrate** $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$

193 Colorless crystals or white crystalline powder. Soluble in water,
 194 and practically insoluble in ethanol (95).