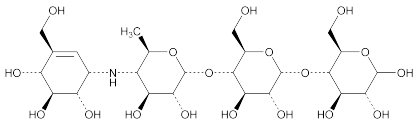
Acarbose



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

D-Glucose, *O*-4,6-dideoxy-4-[[[1*S*-(1α,4α,5*β*,6α)]-4,5,6-trihydroxy-3-(hydroxymethyl)-2-cyclohexen-1-yl]amino]-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-.

O-4,6-Dideoxy-4-[[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)-2-cyclohexen-1-yl]amino]-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-D-glucose [56180-94-0].

» Acarbose is produced by certain strains of *Actinoplanes utahensis*. It contains not less than 95.0 percent and not more than 102.0 percent of $C_{25}H_{43}NO_{18}$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩**—**
USP Acarbose RS
USP Acarbose System Suitability Mixture RS

Identification—

A: *Infrared Absorption* ⟨197K⟩.

B: The retention time of the acarbose peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Specific rotation ⟨781S⟩**:** between +168° and +183°.

Test solution: 10 mg per mL, in water.

pH ⟨791⟩**:** between 5.5 and 7.5, in a solution containing 50 mg per mL.

Water, *Method Ic* ⟨921⟩**:** not more than 4.0%.

Residue on ignition ⟨281⟩**:** not more than 0.2% determined on 1.0 g.

Heavy metals, *Method II* ⟨231⟩**:** 0.002%.

Chromatographic purity—

Mobile phase, System suitability solution, and *Chromatographic system*—Proceed as directed in the *Assay*.

Test solution—Use the *Assay preparation.*

Diluted test solution—Transfer 1.0 mL of the *Test solution* to a 100-mL volumetric flask, dilute with water to volume, and mix.

Procedure—Separately inject equal volumes (about 10 µL) of the *Test solution* and the *Diluted test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each impurity in the portion of Acarbose taken by the formula:

$$(1/F)(r_i / r_A)$$

in which F is the relative response factor for each impurity, as listed in *Table 1; r_i* is the individual peak response for each impurity; and r_A is the response of the main acarbose peak in the chromatogram obtained from the *Diluted test solution*. In addition to not exceeding the limits for each impurity in *Table 1*, not more than 3.0% of total impurities is found.

Assay—

Phosphate buffer—Dissolve 0.6 g of monobasic potassium phosphate and 0.35 g of dibasic sodium phosphate in 900 mL of water, dilute with water to 1 L, and mix.

Mobile phase—Prepare a mixture of acetonitrile and *Phosphate buffer* (750:250). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Reconstitute a vial of USP Acarbose RS in 5.0 mL of water.

System suitability solution—Reconstitute a vial of USP Acarbose System Suitability Mixture RS in 1 mL of water.

Table 1

Name	Approximate Relative Retention Time	Relative Response Factor *(F)*	Limit (%)
Impurity A[1]	0.9	1	0.6
Impurity B[2]	0.8	1.6	0.5
Impurity C[3]	1.2	1	1.5
Impurity D[4]	0.5	1.33	1.0
Impurity E[5]	1.7	0.8	0.2
Impurity F[6]	1.9	0.8	0.3
Impurity G[7]	2.2	0.8	0.3
Impurity H[8]	0.6	1	0.2
Any individual unknown impurity			0.2

[1] *O*-4,6-Dideoxy-4-[[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-D-arabino-hex-2-ulopyranose.

[2] (1*R*,4*R*,5*S*,6*R*)-4,5,6-Trihydroxy-2-(hydroxymethyl)cyclohex-2-enyl 4-*O*-[4,6-dideoxy-4-[[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]-α-D-glucopyranosyl]-α-D-glucopyranoside.

[3] α-D-Glucopyranosyl 4-*O*-[4,6-dideoxy-4-[[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]-α-D-glucopyranosyl]-α-D-glucopyranoside.

[4] 4-*O*-[4,6-Dideoxy-4-[[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]-α-D-glucopyranosyl]-D-glucopyranose.

[5] *O*-4,6-Dideoxy-4-[[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-D-arabino-hex-2-ulopyranose (4-*O*-α-acarbosyl-D-fructopyranose).

[6] *O*-4,6-Dideoxy-4-[[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-D-glucopyranose (4-*O*-α-acarbosyl-D-glucopyranose).

[7] α-D-Glucopyranosyl *O*-4,6-dideoxy-4-[[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranoside (α-D-glucopyranosyl α-acarboside).

[8] *O*-4,6-Dideoxy-4-[[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]-α-D-glucopyranosyl-(1→4)-*O*-6-deoxy-α-D-glucopyranosyl-(1→4)-D-glucopyranose.

Assay preparation—Transfer about 200 mg of Acarbose, accurately weighed, to a 10-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

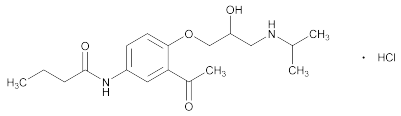
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 210-nm detector and a 4-mm × 25-cm column that contains packing L8. The flow rate is about 2 mL per minute. The column temperature is maintained at 35°. Chromatograph the *System suitability solution,* and identify the acarbose peak and the peaks due to the impurities listed in *Table 1*. Record the peak responses as directed for *Procedure:* the ratio of the height of the impurity A peak to the height of the valley between the impurity A peak and the acarbose peak is not less than 1.2. The chromatogram obtained is similar to the chromatogram supplied with USP Acarbose System Suitability Mixture RS.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for all the peaks. Calculate the quantity, in mg, of $C_{25}H_{43}NO_{18}$ in the portion of Acarbose taken by the formula:

$$10C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Acarbose RS in the *Standard preparation;* and r_U and r_S are the acarbose peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Acebutolol Hydrochloride



$C_{18}H_{28}N_2O_4 \cdot HCl$ 372.89
Butanamide, *N*-[3-acetyl-4-[2-hydroxy-3-[(1-methylethyl)amino]propoxy]phenyl]-, monohydrochloride, (±)-.
(±)-3′-Acetyl-4′-[2-hydroxy-3-(isopropylamino)propoxy]-butyranilide monohydrochloride [34381-68-5].

» Acebutolol Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of $C_{18}H_{28}N_2O_4 \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers, and store at controlled room temperature.

USP Reference standards ⟨11⟩**—**
USP Acebutolol Hydrochloride RS

Identification—

A: *Infrared Absorption* ⟨197K⟩.

B: Prepare a mixture of the *Standard preparation* and the *Assay preparation* (1:1), and chromatograph the mixture as directed in the *Assay:* the chromatogram thus obtained exhibits a single major peak due to acebutolol.

C: It responds to the tests for *Chloride* ⟨191⟩, when tested as directed for alkaloidal hydrochlorides.

pH ⟨791⟩: between 4.5 and 7.0, in a solution (1 in 100).

Melting range ⟨741⟩: between 140° and 144°.

Loss on drying ⟨731⟩**—**Dry it at 105° for 3 hours: it loses not more than 1.0% of its weight.

Residue on ignition ⟨281⟩: not more than 0.1%.

Heavy metals, *Method II* ⟨231⟩: 0.002%.

Chromatographic purity—

Standard solution—Prepare a solution of USP Acebutolol Hydrochloride RS in methanol containing 1.0 mg per mL.

Test solution 1—Prepare a solution of Acebutolol Hydrochloride in methanol containing 10 mg per mL.

Test solution 2—Mix 1 mL of *Test solution 1* and 9 mL of methanol.

Reference solution 1—Transfer 3.0 mL of the *Standard solution* to a 100-mL volumetric flask, dilute with methanol to volume, and mix.

Reference solution 2—Mix 5.0 mL of *Reference solution 1* and 10.0 mL of methanol.

Procedure—Apply separate 20-µL portions of the *Standard solution, Test solution 1, Test Solution 2, Reference solution 1,* and *Reference solution 2* to a suitable thin-layer chromatographic plate (see *Thin-Layer Chromatography* under *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatograms in a solvent system consisting of the upper layer of a mixture of water, butyl alcohol, and glacial acetic acid (50:40:10) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the plate to air-dry. Examine the plate under short-wavelength UV light: the chromatograms from *Test solution 2* and the *Standard solution* show principal spots at about the same R_F value. No secondary spot in the chromatogram from *Test solution 1*, excluding the area at the point of application, is more intense than the principal spot obtained from *Reference solution 1* (0.3%), and not more than two secondary spots in the chromatogram from *Test solution 1* are more intense than the principal spot obtained from *Reference solution 2* (0.1%), and the total of all impurities detected in the chromatogram of *Test solution 1* is not more than 0.5%.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of methanol, a 0.3% aqueous solution of sodium dodecyl sulfate, and glacial acetic acid (675:325:20). Make adjustments if necessary to achieve a retention time for acebutolol of between 4 minutes and 7 minutes (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Quantitatively dissolve an accurately weighed quantity of USP Acebutolol Hydrochloride RS in water to obtain a solution having a known concentration of about 0.14 mg per mL.

Assay preparation—Transfer about 35 mg of Acebutolol Hydrochloride, accurately weighed, to a 250-mL volumetric flask, dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the column efficiency is not less than 1500 theoretical plates; the tailing factor is not more than 2.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_{18}H_{28}N_2O_4 \cdot HCl$ in the portion of Acebutolol Hydrochloride taken by the formula:

$$250C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Acebutolol Hydrochloride RS in the *Standard preparation;* and r_U and r_S are the acebutolol peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.