Impurity A. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 0.40 g of the substance to be examined in *distilled water R* and dilute to 20.0 ml with the same solvent. Dilute 10.0 ml of this solution to 100.0 ml with *borate buffer solution pH 10.4 R*. Place 3.0 ml of the solution obtained in a 25 ml ground-glass-stoppered tube. Add 0.15 ml of a freshly prepared 5 g/l solution of *fluorescamine R* in *acetonitrile R*. Shake immediately and vigorously for 30 s. Place in a water-bath at 50 °C for 30 min. Cool under a stream of cold water. Centrifuge and filter the supernatant through a suitable membrane filter (0.45 µm), 25 mm in diameter.

Reference solution. Dissolve 50 mg of *acamprosate impurity A CRS* in *distilled water R* and dilute to 200.0 ml with the same solvent. Dilute 0.4 ml of the solution to 100.0 ml with *borate buffer solution pH 10.4 R*. Treat 3.0 ml of this solution in the same way as the test solution

Column:

– *size*: *l* = 0.15 m, Ø = 4.6 mm,
– *stationary phase*: *spherical octadecylsilyl silica gel for chromatography R* (5 µm) with a specific surface area of 170 m^2/g and a pore size of 12 nm.

Mobile phase: *acetonitrile R, methanol R, 0.1 M phosphate buffer solution pH 6.5 R* (10:10:80 *V/V/V*).

Flow rate: 1 ml/min.

Detection: spectrophotometer at 261 nm.

Injection: 20 µl.

Run time: 6 times the retention time of impurity A

Retention times: fluorescamine = about 4 min; impurity A = about 8 min; acamprosate is not detected by this system.

Limits:

– *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.05 per cent).

Heavy metals (*2.4.8*): maximum 10 ppm.

Dissolve 2.0 g in *distilled water R* and dilute to 20 ml with the same solvent. 12 ml of the solution complies with limit test A. Prepare the standard using 10 ml of *lead standard solution (1 ppm Pb) R*.

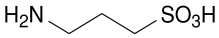
Loss on drying (*2.2.32*): maximum 0.4 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

To 4 g of *cation exchange resin R* (75-150 µm) add 20 ml of *distilled water R* and stir magnetically for 10 min. Introduce this suspension into a glass column, 45 cm long and 2.2 cm in internal diameter, equipped with a polytetrafluoroethylene flow cap covered by a glass-wool plug. Allow a few millilitres of this solution to flow, then place a plug of glass wool over the resin. Pass 50 ml of *1 M hydrochloric acid* through the column. The pH of the eluate is close to 1. Wash with 3 quantities, each of 200 ml, of *distilled water R* to obtain an eluate at pH 6. Dissolve 0.100 g of the substance to be examined in 15 ml of *distilled water R*. Pass through the column and wash with 3 quantities, each of 25 ml, of *distilled water R*, collecting the eluate. Allow to elute until an eluate at pH 6 is obtained. Titrate the solution obtained with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M sodium hydroxide* corresponds to 20.02 mg of $C_{10}H_{20}CaN_2O_8S_2$.

IMPURITIES

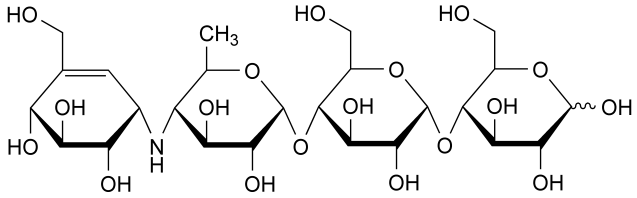


A. 3-aminopropane-1-sulphonic acid (homotaurine).

01/2008:2089

ACARBOSE

Acarbosum



$C_{25}H_{43}NO_{18}$ M_r 646
[56180-94-0]

DEFINITION

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-glucopyranose, which is produced by certain strains of *Actinoplanes utahensis*.

Content: 95.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or yellowish, amorphous powder, hygroscopic.

Solubility: very soluble in water, soluble in methanol, practically insoluble in methylene chloride.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *acarbose for identification CRS*.

B. Examine the chromatograms obtained in the assay.
Results: the principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Solution S. Dissolve 1.00 g in *carbon dioxide-free water R* and dilute to 20.0 ml with the same solvent.

pH (*2.2.3*): 5.5 to 7.5 for solution S.

Specific optical rotation (*2.2.7*): + 168 to + 183 (anhydrous substance).

Dilute 2.0 ml of solution S to 10.0 ml with *water R*.

Absorbance (*2.2.25*): maximum 0.15 at 425 nm for solution S.

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 0.200 g of the substance to be examined in *water R* and dilute to 10.0 ml with the same solvent.

Reference solution (a). Dissolve the contents of a vial of *acarbose CRS* in 5.0 ml of *water R*.

Reference solution (b). Dissolve 20 mg of *acarbose for peak identification CRS* (acarbose containing impurities A, B, C, D, E, F, G and H) in 1 ml of *water R*.

Reference solution (c). Dilute 1.0 ml of the test solution to 100.0 ml with *water R*.

Column:

– *size*: *l* = 0.25 m, Ø = 4 mm,

– *stationary phase*: *aminopropylsilyl silica gel for chromatography R* (5 µm),

– *temperature*: 35 °C.

Mobile phase: mix 750 volumes of *acetonitrile R1* and 250 volumes of a solution containing 0.60 g/l of *potassium dihydrogen phosphate R* and 0.35 g/l of *disodium hydrogen phosphate dihydrate R*.

Flow rate: 2.0 ml/min.

Detection: spectrophotometer at 210 nm.

Injection: 10 µl of the test solution and reference solutions (b) and (c).

Run time: 2.5 times the retention time of acarbose.

Identification of impurities: use the chromatogram supplied with *acarbose for peak identification CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B, C, D, E, F, G and H.

Relative retention with reference to acarbose (retention time = about 16 min): impurity D = about 0.5; impurity H = about 0.6; impurity B = about 0.8; impurity A = about 0.9; impurity C = about 1.2; impurity E = about 1.7; impurity F = about 1.9; impurity G = about 2.2.

System suitability: reference solution (b):

– *peak-to-valley ratio*: minimum 1.2, where H_p = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to acarbose,

– the chromatogram obtained is similar to the chromatogram supplied with *acarbose for peak identification CRS*.

Limits:

– *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity B = 0.63; impurity D = 0.75; impurity E = 1.25; impurity F = 1.25; impurity G = 1.25;

– *impurity A*: not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.6 per cent);

– *impurity B*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent);

– *impurity C*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (1.5 per cent);

– *impurity D*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent);

– *impurity E*: not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent);

– *impurities F, G*: for each impurity, not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent);

– *impurity H*: not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent);

– *any other impurities*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent);

– *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (c) (3.0 per cent);

– *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent).

Heavy metals (*2.4.8*): maximum 20 ppm.

Dissolve 1.5 g in *water R* and dilute to 15 ml with the same solvent. If the solution is not clear, carry out prefiltration and use the filtrate. 10 ml complies with limit test E. Prepare the reference solution using 20 ml of *lead standard solution (1 ppm Pb) R*.

Water (*2.5.12*): maximum 4.0 per cent, determined on 0.300 g.

Sulphated ash (*2.4.14*): maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection: test solution and reference solution (a).

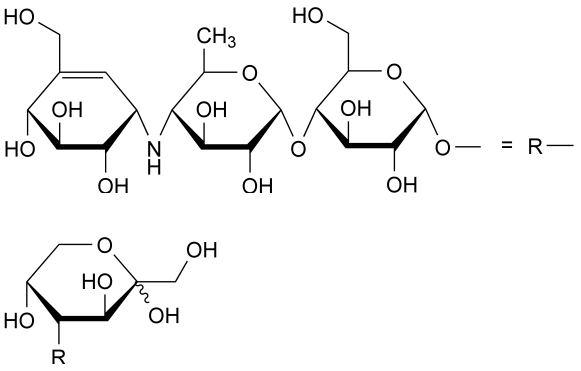
Calculate the percentage content of $C_{25}H_{43}NO_{18}$ from the areas of the peaks and the declared content of *acarbose CRS*.

STORAGE

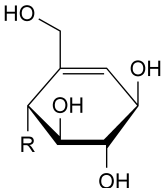
In an airtight container.

IMPURITIES

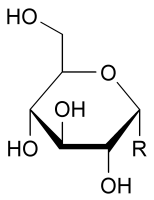
Specified impurities: *A, B, C, D, E, F, G, H.*



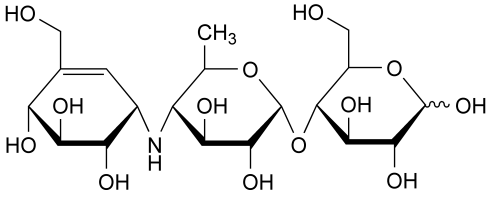
A. *O*-4,6-dideoxy-4-[[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-D-*arabino*-hex-2-ulopyranose,



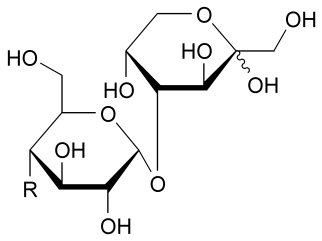
B. (1*R*,4*R*,5*S*,6*R*)-4,5,6-trihydroxy-2-(hydroxymethyl)cyclohex-2-enyl 4-*O*-[4,6-dideoxy-4-[[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]-α-D-glucopyranosyl]-α-D-glucopyranoside,



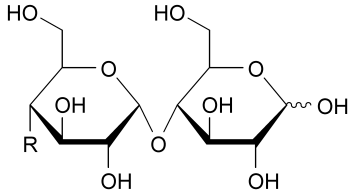
C. α-D-glucopyranosyl 4-*O*-[4,6-dideoxy-4-[[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]-α-D-glucopyranosyl]-α-D-glucopyranoside,



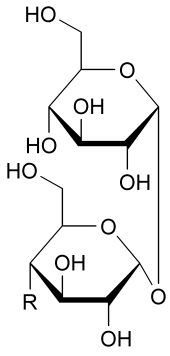
D. 4-*O*-[4,6-dideoxy-4-[[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]-α-D-glucopyranosyl]-D-glucopyranose,



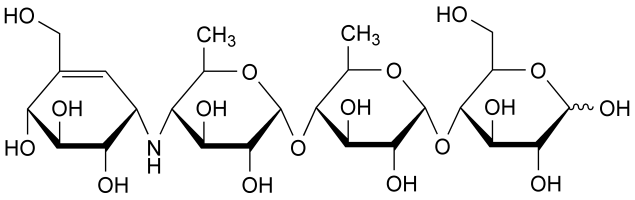
E. *O*-4,6-dideoxy-4-[[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-D-*arabino*-hex-2-ulopyranose (4-*O*-α-acarbosyl-D-fructopyranose),



F. *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),



G. 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),

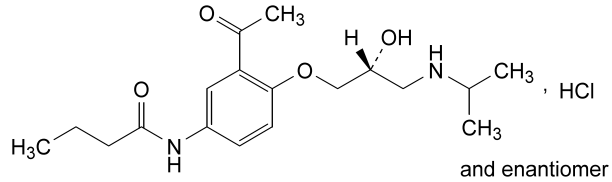


H. *O*-4,6-dideoxy-4-[[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]-α-D-glucopyranosyl-(1→4)-*O*-6-deoxy-α-D-glucopyranosyl-(1→4)-D-glucopyranose.

01/2008:0871
corrected 6.0

ACEBUTOLOL HYDROCHLORIDE

Acebutololi hydrochloridum



and enantiomer

$C_{18}H_{29}ClN_2O_4$ M_r 372.9
[34381-68-5]

DEFINITION

N-[3-Acetyl-4-[(2*RS*)-2-hydroxy-3-[(1-methylethyl)amino]propoxy]phenyl]butanamide hydrochloride.

Content: 99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: freely soluble in water and in ethanol (96 per cent), very slightly soluble in acetone and in methylene chloride.

mp: about 143 °C.

IDENTIFICATION

First identification: B, D.

Second identification: A, C, D.

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution. Dissolve 20.0 mg in a 0.1 per cent *V/V* solution of *hydrochloric acid R* and dilute to 100.0 ml with the same acid solution. Dilute 5.0 ml of this solution to 100.0 ml with a 0.1 per cent *V/V* solution of *hydrochloric acid R*.

Spectral range: 220-350 nm.

Absorption maxima: at 233 nm and 322 nm.

Specific absorbance at the absorption maximum: 555 to 605 at 233 nm.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation: discs.

Comparison: *acebutolol hydrochloride CRS*.

C. Thin-layer chromatography (*2.2.27*).

Test solution. Dissolve 20 mg of the substance to be examined in *methanol R* and dilute to 20 ml with the same solvent.