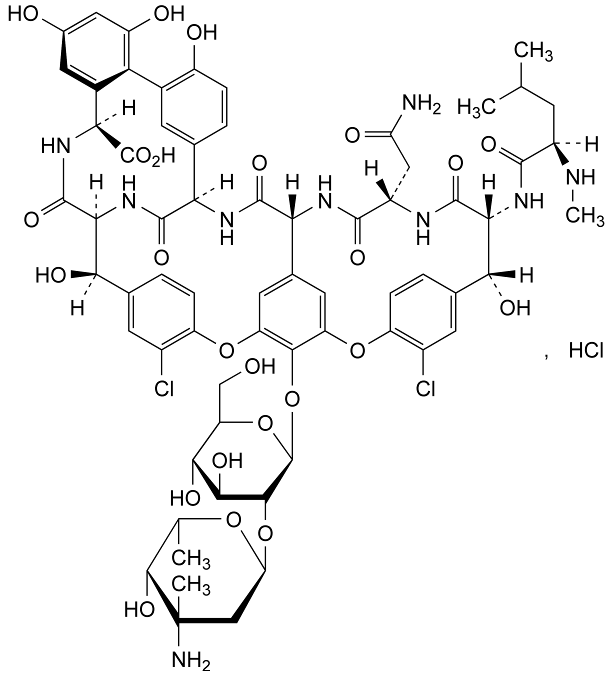
01/2008:1058
corrected 6.0

VANCOMYCIN HYDROCHLORIDE

Vancomycini hydrochloridum



$C_{66}H_{76}Cl_3N_9O_{24}$ M_r 1486

DEFINITION

Hydrochloride of a mixture of related glycopeptides, consisting principally of the monohydrochloride of (3*S*,6*R*,7*R*,22*R*,23*S*,26*S*,30aS_a,36*R*,38a*R*)-3-(2-amino-2-oxoethyl)-44-[[2-*O*-(3-amino-2,3,6-trideoxy-3-*C*-methyl-α-L-*lyxo*-hexopyranosyl)-β-D-glucopyranosyl]oxy]-10,19-dichloro-7,22,28,30,32-pentahydroxy-6-[[(2*R*)-4-methyl-2-(methylamino)pentanoyl]amino]-2,5,24,38,39-pentaoxo-2,3,4,5,6,7,23,24,25,26,36,37,38,38a-tetradecahydro-22*H*-8,11:18,21-dietheno-23,36-(iminomethano)-13,16:31,35-dimetheno-1*H*,13*H*-[1,6,9]oxadiazacyclohexadecino[4,5-*m*][10,2,16]benzoxadiazacyclotetracosine-26-carboxylic acid (vancomycin B).

Substance produced by certain strains of *Amycolatopsis orientalis* or obtained by any other means.

Potency: minimum 1050 IU/mg (anhydrous substance).

CHARACTERS

Appearance: white or almost white, hygroscopic powder.

Solubility: freely soluble in water, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Examine the chromatograms obtained in the test for vancomycin B.

Results: the principal peak in the chromatogram obtained with test solution (a) is similar in retention time to the principal peak in the chromatogram obtained with the reference solution.

B. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Appearance of solution. The solution is clear (*2.2.1*) and its absorbance (*2.2.25*) at 450 nm is not greater than 0.10.

Dissolve 2.50 g in *water R* and dilute to 25.0 ml with the same solvent.

pH (*2.2.3*): 2.5 to 4.5.

Dissolve 0.50 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Vancomycin B. Liquid chromatography (*2.2.29*). *Use the solutions within 4 h of preparation.*

Test solution (a). Dissolve 10.0 mg of the substance to be examined in mobile phase A and dilute to 5.0 ml with mobile phase A.

Test solution (b). Dilute 2.0 ml of test solution (a) to 50.0 ml with mobile phase A.

Test solution (c). Dilute 0.5 ml of test solution (b) to 20.0 ml with mobile phase A.

Reference solution. Dissolve the contents of a vial of *vancomycin hydrochloride CRS* in *water R* and dilute with the same solvent to obtain a solution containing 0.5 mg/ml. Heat at 65 °C for 24 h. Allow to cool.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 μm).

Mobile phase:

- *mobile phase A*: to 4 ml of *triethylamine R* add 1996 ml of *water R* and adjust to pH 3.2 with *phosphoric acid R*; to 920 ml of this solution add 10 ml of *tetrahydrofuran R* and 70 ml of *acetonitrile R*;
- *mobile phase B*: to 4 ml of *triethylamine R* add 1996 ml of *water R* and adjust to pH 3.2 with *phosphoric acid R*; to 700 ml of this solution add 10 ml of *tetrahydrofuran R* and 290 ml of *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 13	100	0
13 - 22	100 → 0	0 → 100
22 - 26	0	100

Flow rate: 1.0 ml/min.

Detection: spectrophotometer at 280 nm.

Injection: 20 μl.

System suitability:

- *resolution*: minimum 5.0 between the 2 principal peaks in the chromatogram obtained with the reference solution;
- *signal-to-noise ratio*: minimum 5 for the principal peak in the chromatogram obtained with test solution (c);
- *symmetry factor*: maximum 1.6 for the peak due to vancomycin in the chromatogram obtained with test solution (b).

Calculate the percentage content of vancomycin B hydrochloride using the following expression:

$$\frac{A_b \times 100}{A_b + \left(\frac{A_t}{25}\right)}$$

A_b = area of the peak due to vancomycin B in the chromatogram obtained with test solution (b);

A_t = sum of the areas of the peaks due to impurities in the chromatogram obtained with test solution (a).

Limit:

- *vancomycin B*: minimum 93.0 per cent.

Related substances. Liquid chromatography (*2.2.29*) as described in the test for vancomycin B with the following modifications.

Injection: test solution (a), (b) and (c).

Calculate the percentage content of each impurity using the following expression:

$$\frac{\left(\frac{A_i}{25}\right) \times 100}{A_b + \left(\frac{A_t}{25}\right)}$$

A_i = area of the peak due to an impurity in the chromatogram obtained with test solution (a);

A_b = area of the peak due to vancomycin B in the chromatogram obtained with test solution (b);

A_t = sum of the areas of the peaks due to impurities in the chromatogram obtained with test solution (a).

Limits:

- *any impurity*: for each impurity, maximum 4.0 per cent;
- *total*: maximum 7.0 per cent;
- *disregard limit*: the area of the principal peak in the chromatogram obtained with test solution (c) (0.1 per cent).

Heavy metals (*2.4.8*): maximum 30 ppm.

1.0 g complies with test C. Prepare the reference solution using 3.0 ml of *lead standard solution (10 ppm Pb) R.*

Water (*2.5.12*): maximum 5.0 per cent, determined on 0.500 g.

Sulphated ash (*2.4.14*): maximum 1.0 per cent, determined on 1.00 g.

Bacterial endotoxins (*2.6.14*): less than 0.25 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

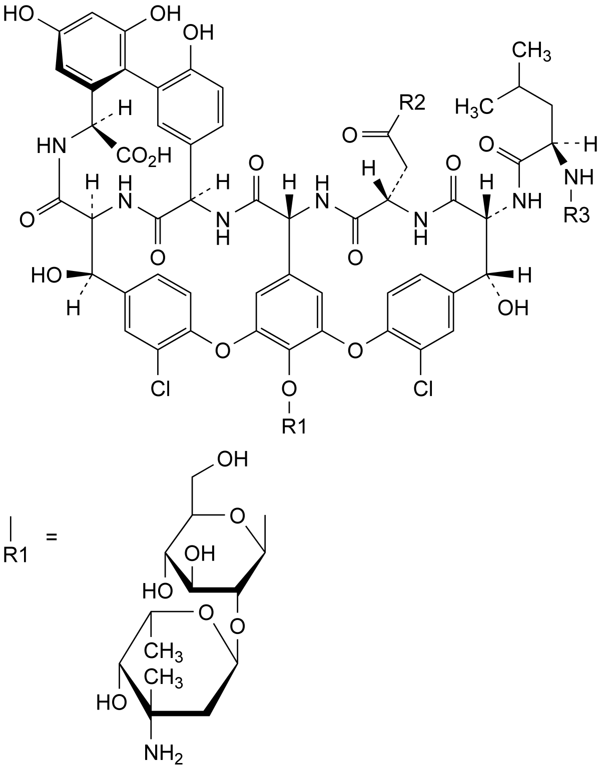
ASSAY

Carry out the microbiological assay of antibiotics (*2.7.2*). Use *vancomycin hydrochloride CRS* as the chemical reference substance.

STORAGE

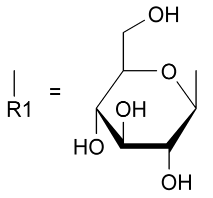
In an airtight container, protected from light. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

IMPURITIES

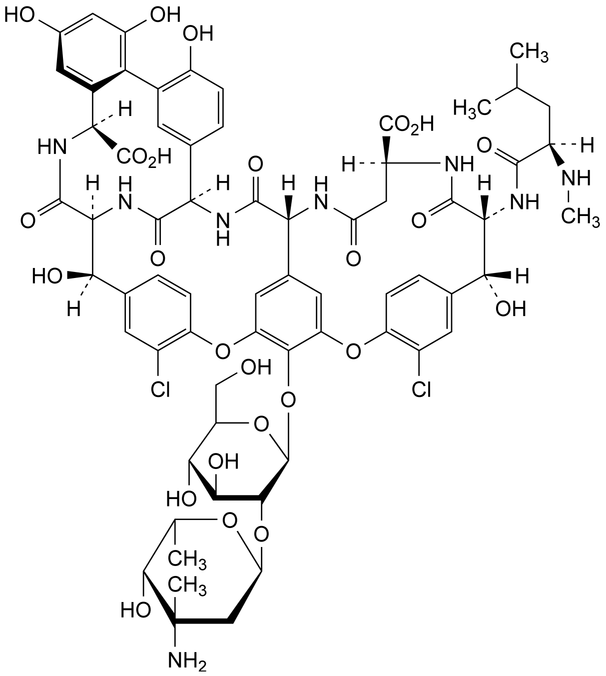


A. R2 = NH_2, R3 = H: *N*-demethylvancomycin B,

C. R1 = H, R2 = NH_2, R3 = CH_3: aglucovancomycin B,



D. R2 = NH_2, R3 = CH_3: desvancosaminylvancomycin B,

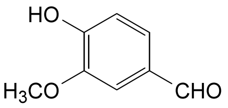


B. (4*S*,7*R*,8*R*,23*R*,24*S*,27*S*,31aS_{a},37*R*,39a*R*)-45-[[2-*O*-(3-amino-2,3,6-trideoxy-3-*C*-methyl-α-L-*lyxo*-hexopyranosyl)-β-D-glucopyranosyl]oxy]-11,20-dichloro-8,23,29,31,33-pentahydroxy-7-[[(2*R*)-4-methyl-2-(methylamino)pentanoyl]amino]-2,6,25,39,40-pentaoxo-1,2,3,4,5,6,7,8,24,25,26,27,37,38,39,39a-hexadecahydro-23*H*-9,12:19,22-dietheno-24,37-(iminomethano)-14,17:32,36-dimetheno-14*H*-[1,6,10]oxadiazacycloheptadecino[4,5-*m*][10,2,16]benzoxadiazacyclotetracosine-4,27-dicarboxylic acid ([β Asp^{3}]vancomycin B).

01/2008:0747

VANILLIN

Vanillinum



$C_8H_8O_3$ M_r 152.1

[121-33-5]

DEFINITION

Vanillin contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 4-hydroxy-3-methoxybenzaldehyde, calculated with reference to the dried substance.

CHARACTERS

White or slightly yellowish, crystalline powder or needles, slightly soluble in water, freely soluble in alcohol and in methanol. It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification: B.

Second identification: A, C, D.

A. Melting point (*2.2.14*): 81 °C to 84 °C.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *vanillin CRS*. Examine the substances prepared as discs.

C. Examine the chromatograms obtained in the test for related substances in daylight after spraying. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. To 5 ml of a saturated solution of the substance to be examined add 0.2 ml of *ferric chloride solution R1*. A blue colour is produced. Heat to 80 °C. The solution becomes brown. On cooling, a white precipitate is formed.

TESTS

Appearance of solution. Dissolve 1.0 g in *alcohol R* and dilute to 20 ml with the same solvent. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution B_6 (*2.2.2, Method II*).

Related substances. Examine by thin-layer chromatography (*2.2.27*), using *silica gel GF*$_{254}$ *R* as the coating substance.

Test solution (a). Dissolve 0.1 g of the substance to be examined in *methanol R* and dilute to 5 ml with the same solvent.

Test solution (b). Dilute 1 ml of test solution (a) to 10 ml with *methanol R*.

Reference solution (a). Dissolve 10 mg of *vanillin CRS* in *methanol R* and dilute to 5 ml with the same solvent.

Reference solution (b). Dilute 0.5 ml of test solution (a) to 100 ml with *methanol R*.

Apply to the plate 5 µl of each solution. Develop in an unsaturated tank over a path of 10 cm using a mixture of 0.5 volumes of *anhydrous acetic acid R*, 1 volume of *methanol R* and 98.5 volumes of *methylene chloride R*. Dry the plate in a current of cold air. Examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). Spray with *dinitrophenylhydrazine-aceto-hydrochloric solution R* and examine in daylight. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

Reaction with sulphuric acid. Dissolve 50 mg in 5 ml of *sulphuric acid R*. After 5 min, the solution is not more intensely coloured than a mixture of 4.9 ml of yellow primary solution and 0.1 ml of red primary solution or a mixture of 4.9 ml of yellow primary solution and 0.1 ml of blue primary solution (*2.2.2, Method I*).

Loss on drying (*2.2.32*). Not more than 1.0 per cent, determined on 1.000 g by drying in a desiccator for 4 h.

Sulphated ash (*2.4.14*). Not more than 0.05 per cent, determined on 2.0 g.

ASSAY

Dissolve 0.120 g in 20 ml of *alcohol R* and add 60 ml of *carbon dioxide-free water R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M sodium hydroxide* is equivalent to 15.21 mg of $C_8H_8O_3$.

STORAGE

Store protected from light.