hydrochloric acid R and 20 mL of *dilute hydrochloric acid R* instead of the solution of the substance to be examined.

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 0.200 g of the substance to be examined in the mobile phase and dilute to 100 mL with the mobile phase.

Reference solution (a). Dissolve 15 mg of *chlorhexidine for performance test CRS* in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (b). Dilute 2.5 mL of the test solution to 100 mL with the mobile phase.

Reference solution (c). Dilute 2.0 mL of reference solution (b) to 10 mL with the mobile phase. Dilute 1.0 mL of this solution to 10 mL with the mobile phase.

Column:

– *size*: *l* = 0.2 m, Ø = 4 mm;

– *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: solution of 2.0 g of *sodium octanesulfonate R* in a mixture of 120 mL of *glacial acetic acid R*, 270 mL of *water R* and 730 mL of *methanol R*.

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 254 nm.

Equilibration: with the mobile phase for at least 1 h.

Injection: 10 µL.

Run time: 6 times the retention time of chlorhexidine.

System suitability: reference solution (a):

– the chromatogram obtained is similar to the chromatogram supplied with *chlorhexidine for performance test CRS* in that the peaks due to impurity A and impurity B precede that due to chlorhexidine; if necessary, adjust the concentration of acetic acid in the mobile phase (increasing the concentration decreases the retention times).

Limits:

– *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.5 per cent);

– *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent); disregard any peak with a relative retention time with reference to chlorhexidine of 0.25 or less.

Loss on drying (*2.2.32*): maximum 3.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

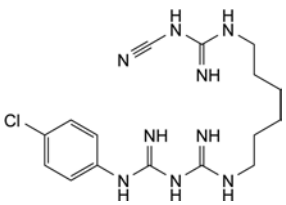
Sulfated ash (*2.4.14*): maximum 0.15 per cent, determined on 1.0 g.

ASSAY

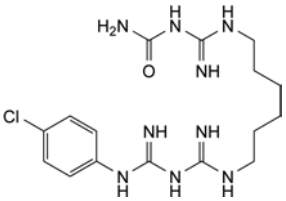
Dissolve 0.140 g in 100 mL of *anhydrous acetic acid R* and titrate with *0.1 M perchloric acid*. Determine the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 15.64 mg of $C_{26}H_{38}Cl_2N_{10}O_4$.

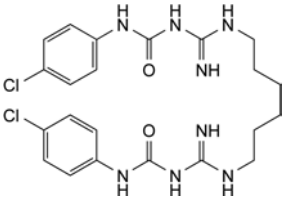
IMPURITIES



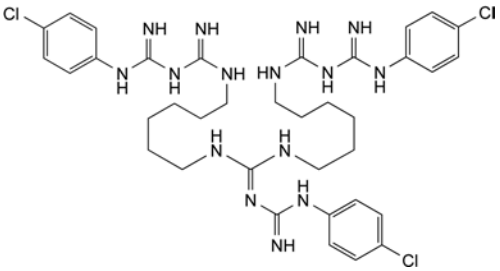
A. 1-(4-chlorophenyl)-5-[6-[(cyanocarbamimidoyl)amino]-hexyl]biguanide,



B. [[6-[[[(4-chlorophenyl)carbamimidoyl]carbamimidoyl]-amino]hexyl]carbamimidoyl]urea,



C. 1,1′-[hexane-1,6-diylbis(iminocarbonimidoyl)]bis[3-(4-chlorophenyl)urea],

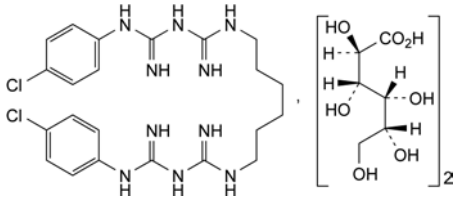


D. 1,1′-[[[[(4-chlorophenyl)carbamimidoyl]imino]methylene]-bis[imino(hexane-1,6-diyl)]]bis[5-(4-chlorophenyl)biguanide].

01/2008:0658
corrected 7.0

CHLORHEXIDINE DIGLUCONATE SOLUTION

Chlorhexidini digluconatis solutio



$C_{34}H_{54}Cl_2N_{10}O_{14}$ M_r 898
[18472-51-0]

DEFINITION

Aqueous solution of 1,1′-(hexane-1,6-diyl)bis[5-(4-chlorophenyl)biguanide] di-D-gluconate.

Content: 190 g/L to 210 g/L.

CHARACTERS

Appearance: almost colourless or pale-yellowish liquid.

Solubility: miscible with water, with not more than 3 parts of acetone and with not more than 5 parts of ethanol (96 per cent).

IDENTIFICATION

First identification: A, B.

Second identification: B, C, D.

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation: to 1 mL add 40 mL of *water R*, cool in iced water, make alkaline to *titan yellow paper R* by adding dropwise, and with stirring, *strong sodium hydroxide solution R* and add 1 mL in excess. Filter, wash the

precipitate with *water R* until the washings are free from alkali and recrystallise from *ethanol (70 per cent V/V) R*. Dry at 100-105 °C. Examine the residue.

Comparison: *chlorhexidine CRS*.

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

Test solution. Dilute 10.0 mL of the preparation to be examined to 50 mL with *water R*.

Reference solution. Dissolve 25 mg of *calcium gluconate CRS* in 1 mL of *water R*.

Plate: *TLC silica gel G plate R*.

Mobile phase: *concentrated ammonia R, ethyl acetate R, water R, ethanol (96 per cent) R* (10:10:30:50 *V/V/V/V*).

Application: 5 µL.

Development: over a path of 10 cm.

Drying: at 100 °C for 20 min and allow to cool.

Detection: spray with a 50 g/L solution of *potassium dichromate R* in a 40 per cent *m/m* solution of *sulfuric acid R*.

Results: after 5 min, the principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

C. To 1 mL add 40 mL of *water R*, cool in iced water, make alkaline to *titan yellow paper R* by adding dropwise, and with stirring, *strong sodium hydroxide solution R* and add 1 mL in excess. Filter, wash the precipitate with *water R* until the washings are free from alkali and recrystallise from *ethanol (70 per cent V/V) R*. Dry at 100-105 °C. The residue melts (*2.2.14*) at 132 °C to 136 °C.

D. To 0.05 mL add 5 mL of a 10 g/L solution of *cetrimide R*, 1 mL of *strong sodium hydroxide solution R* and 1 mL of *bromine water R*; a deep red colour is produced.

TESTS

Relative density (*2.2.5*): 1.06 to 1.07.

pH (*2.2.3*): 5.5 to 7.0.

Dilute 5.0 mL to 100 mL with *carbon dioxide-free water R*.

Chloroaniline: maximum 0.25 per cent, calculated with reference to chlorhexidine digluconate at a nominal concentration of 200 g/L.

Dilute 2.0 mL to 100 mL with *water R*. To 10 mL of this solution add 2.5 mL of *dilute hydrochloric acid R* and dilute to 20 mL with *water R*. Add rapidly and with thorough mixing after each addition: 0.35 mL of *sodium nitrite solution R*, 2 mL of a 50 g/L solution of *ammonium sulfamate R*, 5 mL of a 1 g/L solution of *naphthyle thylenediamine dihydrochloride R*, 1 mL of *ethanol (96 per cent) R*; dilute to 50.0 mL with *water R* and allow to stand for 30 min. Any reddish-blue colour in the solution is not more intense than that in a standard prepared at the same time and in the same manner using a mixture of 10.0 mL of a 0.010 g/L solution of *chloroaniline R* in *dilute hydrochloric acid R* and 10 mL of *water R* instead of the dilution of the preparation to be examined.

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dilute 5.0 mL of the preparation to be examined to 50.0 mL with the mobile phase. Dilute 5.0 mL of this solution to 50.0 mL with the mobile phase.

Reference solution (a). Dissolve 15 mg of *chlorhexidine for performance test CRS* in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (b). Dilute 3.0 mL of the test solution to 100 mL with the mobile phase.

Reference solution (c). Dilute 1.0 mL of reference solution (b) to 50 mL with the mobile phase.

Column:

– *size*: *l* = 0.2 m, Ø = 4 mm;

– *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: solution of 2.0 g of *sodium octanesulfonate R* in a mixture of 120 mL of *glacial acetic acid R*, 270 mL of *water R* and 730 mL of *methanol R*.

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 254 nm.

Equilibration: with the mobile phase for at least 1 hour.

Injection: 10 µL.

Run time: 6 times the retention time of chlorhexidine.

System suitability: reference solution (a):

– the chromatogram obtained is similar to the chromatogram supplied with *chlorhexidine for performance test CRS* in that the peaks due to impurity A and impurity B precede that due to chlorhexidine; if necessary, adjust the concentration of acetic acid in the mobile phase (increasing the concentration decreases the retention times).

Limits:

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

– *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c) (0.06 per cent); disregard any peak with a relative retention time with reference to the principal peak of 0.25 or less.

ASSAY

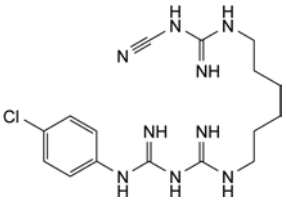
Determine the density (*2.2.5*) of the preparation to be examined. Transfer 1.00 g to a 250 mL beaker and add 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*. Determine the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 22.44 mg of $C_{34}H_{54}Cl_2N_{10}O_{14}$.

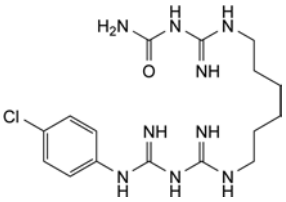
STORAGE

Protected from light.

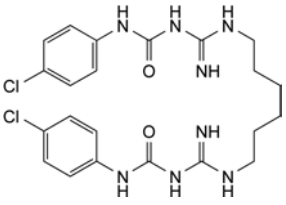
IMPURITIES



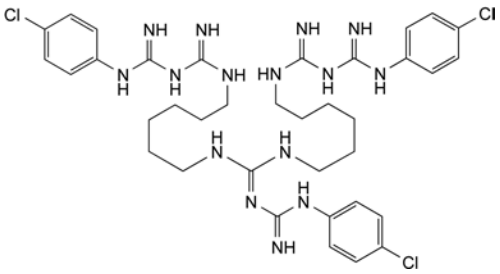
A. 1-(4-chlorophenyl)-5-[6-[(cyanocarbamimidoyl)amino]-hexyl]biguanide,



B. [[6-[[[(4-chlorophenyl)carbamimidoyl]carbamimidoyl]-amino]hexyl]carbamimidoyl]urea,



C. 1,1′-[hexane-1,6-diylbis(iminocarbonimidoyl)]bis[3-(4-chlorophenyl)urea],

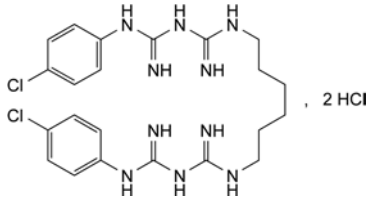


D. 1,1′-[[[[(4-chlorophenyl)carbamimidoyl]imino]methylene]-bis[imino(hexane-1,6-diyl)]]bis[5-(4-chlorophenyl)biguanide].

01/2008:0659
corrected 7.0

CHLORHEXIDINE DIHYDROCHLORIDE

Chlorhexidini dihydrochloridum



$C_{22}H_{32}Cl_4N_{10}$ M_r 578.4
[3697-42-5]

DEFINITION

1,1′-(Hexane-1,6-diyl)bis[5-(4-chlorophenyl)biguanide] dihydrochloride.

Content: 98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: sparingly soluble in water and in propylene glycol, very slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification: A, D.

Second identification: B, C, D.

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *chlorhexidine dihydrochloride CRS*.

B. Dissolve about 5 mg in 5 mL of a warm 10 g/L solution of *cetrimide R* and add 1 mL of *strong sodium hydroxide solution R* and 1 mL of *bromine water R*. A dark red colour is produced.

C. Dissolve 0.3 g in 10 mL of a mixture of equal volumes of *hydrochloric acid R* and *water R*. Add 40 mL of *water R*, filter if necessary and cool in iced water. Make alkaline to *titan yellow paper R* by adding dropwise, and with stirring, *strong sodium hydroxide solution R* and add 1 mL in excess. Filter, wash the precipitate with *water R* until the washings are free from alkali and recrystallise from *ethanol (70 per cent V/V) R*. Dry at 100-105 °C. The residue melts (*2.2.14*) at 132 °C to 136 °C.

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

TESTS

Chloroaniline: maximum 500 ppm.

To 0.20 g add 1 mL of *hydrochloric acid R*, shake for about 30 s, dilute to 30 mL with *water R* and shake until a clear solution is obtained. Add rapidly and with thorough mixing after each addition: 2.5 mL of *dilute hydrochloric acid R*, 0.35 mL of *sodium nitrite solution R*, 2 mL of a 50 g/L solution of *ammonium sulfamate R*, 5 mL of a 1.0 g/L solution of *naphthylethylenediamine dihydrochloride R* and 1 mL of *ethanol (96 per cent) R*; dilute to 50.0 mL with *water R* and allow to stand for 30 min. Any reddish-blue colour in the solution is not more intense than that in a standard prepared at the same time and in the same manner using a mixture of 10.0 mL of a 0.010 g/L solution of *chloroaniline R* in *dilute hydrochloric acid R* and 20 mL of *dilute hydrochloric acid R* instead of the solution of the substance to be examined.

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 0.200 g of the substance to be examined in the mobile phase and dilute to 100 mL with the mobile phase.

Reference solution (a). Dissolve 15 mg of *chlorhexidine for performance test CRS* in the mobile phase and dilute to 10.0 mL with the mobile phase.

Reference solution (b). Dilute 2.5 mL of the test solution to 100 mL with the mobile phase.

Reference solution (c). Dilute 2.0 mL of reference solution (b) to 10 mL with the mobile phase. Dilute 1.0 mL of this solution to 10 mL with the mobile phase.

Column:
- *size*: *l* = 0.2 m, Ø = 4 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: solution of 2.0 g of *sodium octanesulfonate R* in a mixture of 120 mL of *glacial acetic acid R*, 270 mL of *water R* and 730 mL of *methanol R*.

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 254 nm.

Equilibration: with the mobile phase for at least 1 h.

Injection: 10 µL.

Run time: 6 times the retention time of chlorhexidine.

System suitability: reference solution (a):
- the chromatogram obtained is similar to the chromatogram supplied with *chlorhexidine for performance test CRS* in that the peaks due to impurity A and impurity B precede that due to chlorhexidine; if necessary, adjust the concentration of acetic acid in the mobile phase (increasing the concentration decreases the retention times).

Limits:
- *total*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.5 per cent);
- *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent); disregard any peak with a relative retention time with reference to chlorhexidine of 0.25 or less.

Loss on drying (*2.2.32*): maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

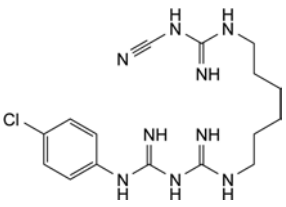
Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 100.0 mg in 5 mL of *anhydrous formic acid R* and add 70 mL of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M perchloric acid* is equivalent to 14.46 mg of $C_{22}H_{32}Cl_4N_{10}$.

IMPURITIES



A. 1-(4-chlorophenyl)-5-[6-[(cyanocarbamimidoyl)amino]-hexyl]biguanide,