Chlorhexidine Gluconate Solution

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Manufacturers, regulatory authorities, health authorities, researchers, and other stakeholders are invited to provide their feedback and comments on this draft proposal. Manufacturers are also invited to submit samples of their products to the IPC to ensure that the proposed monograph adequately controls the quality of the product(s) they manufacture. Comments and samples received after the last date will not be considered by the IPC before finalizing the monograph.

Please send any comments you may have on this draft document to lab.ipc@gov.in, with a copy to Dr. Gaurav Pratap Singh (email: gpsingh.ipc@gov.in) before the last date for comments.

Document History and Schedule for the Adoption Process

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Further follow-up action as required.	

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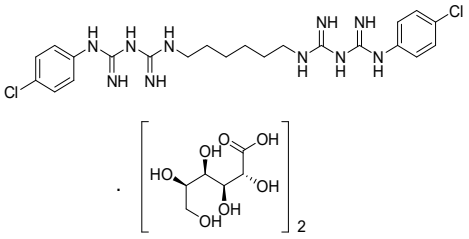
Change **to**: **Chlorhexidine Gluconate Solution**



$C_{22}H_{30}Cl_2N_{10}\cdot 2C_6H_{12}O_7$ Mol. Wt. 897.8

Chlorhexidine Gluconate Solution is an aqueous solution of 1,1'-hexamethylenebis [5-(4-chlorophenyl)biguanide] digluconate.

Chlorhexidine Gluconate Solution contains not less than 19.0 per cent w/v and not more than 21.0 per cent w/v of $C_{22}H_{30}Cl_2N_{10},2C_6H_{12}O_7$.

Category. Antiseptic.

Description. An almost colourless or pale yellowish, clear or slightly opalescent liquid.

Identification

A. To 2 ml, add 80 ml of *water*, cool in ice, add *5 M sodium hydroxide* dropwise with stirring until the solution is slightly alkaline to titan yellow paper and add 2 ml in excess. Filter, wash the precipitate with water until the washings are free from alkali, dissolve it in about 25 ml of *ethanol* on a boiling water-bath and heat until the volume is reduced to about 5 ml. Cool in ice, induce crystallisation, if necessary, by scratching the side of the vessel with a glass rod, filter and dry the crystals at 105°. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *chlorhexidine IPRS* or with the reference spectrum of chlorhexidine. Examine the substance as a dispersion in *potassium bromide IR* without excessive grinding.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

pH (2.4.24).5.5 to 7.0, determined in a solution obtained by diluting 5 ml to 100 ml.

***p*-Chloroaniline**. Not more than 500 PPM.

Determine by liquid chromatography (2.4.14).

Solvent mixture. Dissolve 13.8 g of *sodium dihydrogen orthophosphate* in 750 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid* and dilute to 1000 ml with *water.*

Test solution. Dilute 5.0 ml of the solution to 100.0 ml with *water*. Dilute 10.0 ml of the solution to 250.0 ml with the solvent mixture.

Reference solution (a). A 0.0001 per cent w/v solution of *p-chloroaniline IPRS* in the solvent mixture.

Reference solution (b). A solution containing 0.005 per cent w/v of *chlorhexidine acetate IPRS* and 0.0001 per cent w/v of *p-chloroaniline IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with base-deactivated octadecylsilane bonded to porous silica (5 µm) (Such as Symmetry C18),
- column temperature: 40°,
- mobile phase: A. a mixture of 70 volumes of a buffer solution prepared by dissolving 13.8 g of *sodium dihydrogen orthophosphate* and 5 ml of *triethylamine* in 750 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid* and dilute to 1000 ml with *water,* and 30 volumes of *acetonitrile,*
  B. *acetonitrile*,
- a gradient programme using the conditions given below,

– flow rate: 1.5 ml per minute,
– spectrophotometer set at 239 nm,
– injection volume: 50 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
9	100	0
10	45	55
15	45	55
16	100	0
21	100	0

The relative retention time with reference to chlorhexidine for *p*-chloroaniline is about 1.3.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to chlorhexidine and *p*-chloroaniline is not less than 3.0 and the relative standard deviation for replicate injections is not more than 1.0 per cent, for chlorhexidine peak and not more than 5.0 per cent, for *p*- chloroaniline peak.

Inject reference solution (a) and the test solution.

Calculate the content of *p*-chloroaniline.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE – Store the solutions at a temperature not more than 12°.

Test solution. Dilute 1.0 ml of the solution to 100.0 ml with mobile phase A.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A.

Reference solution (b). A 0.5 per cent w/v solution of *chlorhexidine system suitability mixture IPRS* in mobile phase A.

Chromatographic system
– a stainless steel column 25 cm x 4.6 mm, packed with base-deactivated octadecylsilane bonded to porous silica (5 µm) (Such as Symmetry C18),
– sample temperature: 12°,
– mobile phase: A. a mixture of 20 volumes of a 0.1 per cent v/v solution of *trifluoroacetic acid* in *acetonitrile* and 80 volumes of a 0.1 per cent v/v solution of *trifluoroacetic acid* in *water,*
B. a mixture of 90 volumes of a 0.1 per cent v/v solution of *trifluoroacetic acid* in *acetonitrile* and 10 volumes of a 0.1 per cent v/v solution of *trifluoroacetic acid* in *water,*
– a gradient programme using the conditions given below,
– flow rate: 1 ml per minute,
– spectrophotometer set at 254 nm,
– injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
2	100	0
32	80	20
37	80	20
47	70	30
54	70	30
55	100	0
60	100	0

Name	Relative retention time
Chlorhexidine oxazinone analog[1]	0.23
specified unidentified impurity 1	0.24
Chlorhexidine amine[2]	0.25
Chlorhexidine guanidine[3]	0.35

Chlorhexidine urea[4]	0.36
p-Chlorophenyl urea[5]	0.5
Chlorhexidine nitrile [6]	0.6
Chlorhexidine dimer [7]	0.85
o-Chlorhexidine[8]* and specified unidentified impurity 2a*	0.90, 091
Chlorhexidine glucityl triazine[9]	0.96
Chlorhexidine	1.0
Oxochlorhexidine[10]	1.4

*If present, *o*-chlorhexidine and specified unidentified impurity 2 may not be completely resolved by the method. These peaks are integrated together to determine conformance

[1](5*R*,6*S*)-2-[(4-Chlorophenyl)amino]-5-hydroxy-6-[(1*R*,2*R*)-1,2,3-trihydroxypropyl]-5,6-dihydro-4*H*-1,3-oxazin-4-one,

[2]1-(6-Aminohexyl)-5-(4-chlorophenyl)biguanide,

[3]1-[6-(Carbamimidoylamino)hexyl]-5-(4-chlorophenyl)biguanide,

[4]*N*-{[6-({[(4-Chlorophenyl)carbamimidoyl]carbamimidoyl}amino)hexyl]carbamimidoyl}urea,

[5]1-(4-Chlorophenyl)urea,

[6]1-(4-Chlorophenyl)-5-[6-[(cyanocarbamimidoyl)amino]hexyl]biguanide,

[7]1,5-Bis[5-(4-chlorophenyl)biguanidylhexyl]biguanide,

[8]1-(2-Chlorophenyl)-5-[6-({[(4-chloro phenyl)carbamimidoyl]carbamimidoyl}amino) hexyl]biguanide,

[9]1-(4-Chlorophenyl)-5-[6-({4-[(4-chlorophenyl)amino]-6-[(1*S*,2*R*,3*R*,4*R*)-1,2,3,4,5-pentahydroxypentyl]-1,3,5-triazin-2-yl}amino)hexyl]biguanide,

[10]*N*-(4-Chlorophenyl)-*N'*-{[6-({[(4-chlorophenyl)carbamimidoyl]carbamimidoyl}amino)hexyl]carbamimidoyl}urea.

Inject reference solution (b). The test is not valid unless the peak-to-valley ratio is not less than 2.0 between the peak due to chlorhexidine urea and chlorhexidine guanidine.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to chlorhexidine oxazinone analog, specified unidentified impurity 1, chlorhexidine urea and p-chlorophenyl urea, each of, is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent), the area of the peak corresponding to chlorhexidine nitrile, *o*-chlorhexidine + specified unidentified impurity 2, chlorhexidine glucityl triazine and oxochlorhexidine is not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent), the area of any peak corresponding to chlorhexidine amine is not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent), the area of any peak corresponding to chlorhexidine guanidine is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent), the area of any peak corresponding to chlorhexidine dimer is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent), the area of any other secondary peak is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent) and the sum of the areas of all the secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (3.0 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Assay. Determine by liquid chromatography (2.4.14),

Test solution. Dilute 5.0 ml of the solution to 250.0 ml with *water*. Dilute 5.0 ml of the solution to 250.0 ml with mobile phase A.

Reference solution (a). A 0.005 per cent w/v solution of *chlorhexidine acetate IPRS* in mobile phase A.

Reference solution (b). A solution containing 0.005 per cent w/v of *chlorhexidine acetate IPRS* and 0.0001 per cent w/v of *p-chloroaniline IPRS* in mobile phase A.

Use chromatographic system as described under *p*-Chloroaniline.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to chlorhexidine and *p*-chloroaniline is not less than 3.0 and the relative standard deviation for replicate injections is not more than 1.0 per cent, for chlorhexidine peak and not more than 5.0 per cent, for *p*-chloroaniline peak.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{22}H_{30}Cl_2N_{10},2C_6H_{12}O_7$.

Storage. Store protected from light, at a temperature not exceeding 30°.