Chlorhexidine Hydrochloride

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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.	

Chlorhexidine Hydrochloride. Page 1843

Change to: **Chlorhexidine Hydrochloride**

Chlorhexidine Dihydrochloride

$C_{22}H_{30}Cl_2N_{10}$,2HCl Mol. Wt. 578.4

Chlorhexidine Hydrochloride is 1,1'-(hexane-1,6-diyl)bis[5-(4-chlorophenyl)biguanide] dihydrochloride.

Chlorhexidine Hydrochloride contains not less than 98.0 per cent and not more than 101.0 per cent of chlorhexidine dihydrochloride, $C_{22}H_{30}Cl_2N_{10}$, 2HCl calculated on the dried basis.

Category. Antiseptic.

Description. A white or almost white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *chlorhexidine hydrochloride IPRS* or with the reference spectrum of chlorhexidine hydrochloride.

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

C. It gives reaction (A) of chlorides (2.3.1).

Tests

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

Determine by liquid chromatography (2.4.14).

Test solution (*a).* Dissolve 0.2 g of the substance under examination in mobile phase A and dilute to 100.0 ml of mobile phase A.

Test solution (b). Dilute 5.0 ml of test solution (a) to 200.0 ml with mobile phase A.

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

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

Reference solution (c). 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.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with base-deactivated octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: A. a mixture of 70 volume 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,* 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 (c). 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 test solution (a).

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. Dissolve 0.14 g of the substance under examination in mobile phase A and dilute to 100.0 ml of 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 Luna C18 (2)),
- 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 guanidine[1]	0.35
Chlorhexidine nitrile [2]	0.6
Chlorhexidine dimer [3]	0.85
o-Chlorhexidine[4]* and specified unidentified impurity 2[5]*	0.90, 0.91
Chlorhexidine	1.0
Oxochlorhexidine[6]	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]1-[6-(Carbamimidoylamino)hexyl]-5-(4-chlorophenyl)biguanide,

[2]1-(4-Chlorophenyl)-5-[6-[(cyanocarbamimidoyl)amino]hexyl]biguanide,

[3]1,5-Bis[5-(4-chlorophenyl)biguanidylhexyl]biguanide,

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

[5]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,

[6]*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 guanidine and chlorhexidine nitrile, each of, is not more than 0.15 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent), the area of any peak corresponding to chlorhexidine dimer 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 sum of the areas of the peaks corresponding to *o*-chlorhexidine + specified unidentified impurity 2 and oxochlorhexidine, each of, 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 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 the area of the principal peak in the chromatogram with reference solution (a) (1.0 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).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Determine by liquid chromatography (2.4.14), as described under *p*-Chloroaniline with following modification.

Inject reference solution (c). 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 (b) and test solution (b).

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

1 mg of chlorhexidine acetate, $C_{22}H_{30}Cl_2N_{10},2C_2H_4O_2$ is equivalent to 0.925 mg of chlorhexidine hydrochloride.

Insert at the end
Storage. Store protected from light and moisture, at a temperature not exceeding 30°