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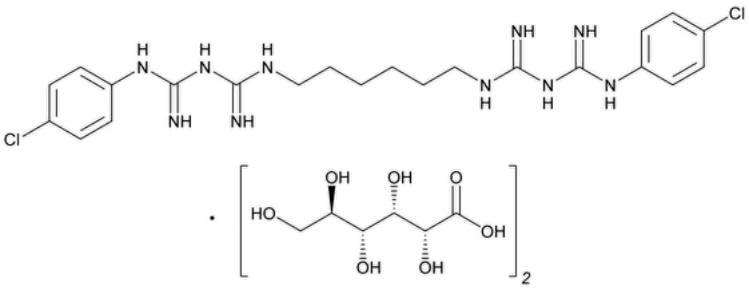
Chlorhexidine Gluconate Solution

Change to read:



$C_{22}H_{30}Cl_2N_{10} \cdot 2C_6H_{12}O_7$ 897.76

2,4,11,13-Tetraazatetradecanediimidamide, *N*,*N*^{2}-bis(4-chlorophenyl)-3,12-diimino-, di-D-gluconate;

1,1'-Hexamethylenebis[5-(4-chlorophenyl)biguanide] di-D-gluconate CAS RN®: 18472-51-0; UNII: MOR84MUD8E.

▲Chlorhexidine (free base)

$C_{22}H_{30}Cl_2N_{10}$ 505.45 CAS RN®: 55-56-1; UNII: R4KO0DY52L.▲ (USP 1-May-2022)

DEFINITION

Chlorhexidine Gluconate Solution is an aqueous solution of chlorhexidine gluconate. It contains NLT 19.0% and NMT 21.0% (w/v) of chlorhexidine gluconate ($C_{22}H_{30}Cl_2N_{10} \cdot 2C_6H_{12}O_7$).

IDENTIFICATION

• **A. Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*:** 197K

Standard: 5 mg/mL of USP Chlorhexidine RS in 70% alcohol. Recrystallize this solution, and dry the crystals at 105° for 1 h.

Sample: To 1 mL of Chlorhexidine Gluconate Solution add 40 mL of water, and cool in ice. Add 10 N sodium hydroxide, dropwise with stirring, until the solution produces a red color on thiazol yellow paper, and add 1 mL in excess. Filter, wash the precipitate with water until the washings are free from alkali, recrystallize the residue from 70% alcohol, and dry the crystals at 105° for 1 h.

Acceptance criteria: Meets the requirements

• **B. Thin-Layer Chromatographic Identification Test ⟨201⟩**

Standard solution: 20 mg/mL of USP Potassium Gluconate RS

Sample solution: Dilute 10 mL of Chlorhexidine Gluconate Solution with water to 50 mL. This solution contains 40 mg/mL of chlorhexidine gluconate.

Chromatographic system

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 5 µL

Developing solvent system: Alcohol, ethyl acetate, ammonium hydroxide, and water (5:1:1:3)

Spray reagent: Dissolve 2.5 g of ammonium molybdate in 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask. Add 1.0 g of ceric sulfate, swirl to dissolve, and dilute with 2 N sulfuric acid to volume.

Analysis

Samples: *Standard solution* and *Sample solution*

Develop the chromatogram in a solvent system until the solvent front has moved 10 cm from the point of spotting. Remove the plate from the chamber, and dry at 110° for 20 min. Allow to cool, and spray with *Spray reagent*. Heat the plate at 110° for 10 min.

Acceptance criteria: The principal spot of the *Sample solution* corresponds in color, size, and R_F value to that of the *Standard solution*.

Add the following:

▲• **C.** The retention time of the major peak from the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2022)

ASSAY

Change to read:

• **Procedure**

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Solution A: Dissolve 27.6 g of monobasic sodium phosphate and 10 mL of triethylamine in 1.5 L of water. Adjust with phosphoric acid to a pH of 3.0, and dilute with water to 2000 mL. Mix the resulting solution with acetonitrile (70:30).

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
9	100	0
10	45	55
15	45	55
16	100	0
21	100	0

System suitability solution: 50 µg/mL of USP Chlorhexidine Acetate RS and 1 µg/mL of USP *p*-Chloroaniline RS in *Solution A*

Standard solution: 50 µg/mL of USP Chlorhexidine Acetate RS in *Solution A*

Sample stock solution: Transfer 5.0 mL of Chlorhexidine Gluconate Solution to a 250-mL volumetric flask, and dilute with water to volume.

Sample solution: Transfer 5.0 mL of the *Sample stock solution* to a 250-mL volumetric flask, and dilute with *Solution A*.

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 239 nm

Column: 4.6-mm × 25-cm; base-deactivated 5-µm packing L1

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 50 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for chlorhexidine and *p*-chloroaniline are about 1.0 and 1.3, respectively.]

Suitability requirements

Resolution: NLT 3.0 between chlorhexidine and *p*-chloroaniline

Relative standard deviation: NMT 0.73% for chlorhexidine and NMT 5.0% for *p*-chloroaniline

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage (w/v) of chlorhexidine gluconate ($C_{22}H_{30}Cl_2N_{10} \cdot 2C_6H_{12}O_7$) in the portion of Chlorhexidine Gluconate Solution taken:

$$\text{Result} = (r_U/r_S) \times (0.25 \times C_S) \times (M_{r1}/M_{r2})$$

r_U = peak response of chlorhexidine from the *Sample solution*

r_S = peak response of chlorhexidine from the *Standard solution*

C_S = concentration of USP Chlorhexidine Acetate RS in the *Standard solution* (µg/mL)

M_{r1} = molecular weight of chlorhexidine gluconate, 897.76

M_{r2} = molecular weight of chlorhexidine acetate, ▲625.56▲ (USP 1-May-2022)

Acceptance criteria: 19.0%–21.0% (w/v)

IMPURITIES

• **ORGANIC IMPURITIES**

Store the *System suitability solution*, the *Sample solution*, and the *Diluted sample solution* at a temperature of NMT 12°.

Solution A: 0.1% (v/v) trifluoroacetic acid in acetonitrile

Solution B: 0.1% (v/v) trifluoroacetic acid in water

Solution C: *Solution A* and *Solution B* (20:80)

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Solution D: *Solution A* and *Solution B* (90:10)

Mobile phase: See *Table 2*. Return to original conditions, and equilibrate the system.

Table 2

Time (min)	Solution C (%)	Solution D (%)
0	100	0
2	100	0
32	80	20
37	80	20
47	70	30
54	70	30

System suitability solution: 5.0 mg/mL of USP Chlorhexidine System Suitability Mixture RS in *Solution C*

Sample solution: Dilute 1.0 mL of Chlorhexidine Gluconate Solution with *Solution C* to 100.0 mL.

Diluted sample solution: Dilute 1.0 mL of *Sample solution* with *Solution C* to 100.0 mL.

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; base-deactivated 5-µm packing L1

Temperatures

Autosampler: NMT 12°

Column: 30°

Flow rate: 1.0 mL/min

Injection volume: 10 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Peak-to-valley ratio: NLT 2.0 between chlorhexidine urea and chlorhexidine guanidine

Resolution: NLT 3.0 between chlorhexidine oxazinone analog and chlorhexidine amine

Analysis

Samples: *Sample solution* and *Diluted sample solution*

Calculate the percentage of each impurity in the portion of Chlorhexidine Gluconate Solution taken:

$$\text{Result} = (r_U/r_S) \times D \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of chlorhexidine from the *Diluted sample solution*

D = dilution factor used to prepare the *Diluted sample solution*, 0.01

Acceptance criteria: See *Table 3*. The reporting threshold is 0.05%.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Chlorhexidine oxazinone analog	0.23	0.2
Specified unidentified impurity 1	0.24	0.2
Chlorhexidine amine	0.25	0.3
Chlorhexidine guanidine	0.35	1.0

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Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Chlorhexidine urea	0.36	0.2
p-Chlorophenyl urea	0.5	0.2
Chlorhexidine nitrile	0.6	0.4
Chlorhexidine dimer	0.85	0.5
o-Chlorhexidine and specified unidentified impurity 2	0.90–0.91	0.4[a]
Chlorhexidine glucityl triazine	0.96	0.4
Chlorhexidine	1.0	—
Oxochlorhexidine	1.4	0.4
Any individual unspecified impurity	—	0.10
Total impurities	—	3.0

[a] 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.

Change to read:

• **Limit of *p*-Chloroaniline**

Solution A, Solution B, Mobile phase, System suitability solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Diluent: 27.6 g of monobasic sodium phosphate in 1.5 L of water. Adjust with phosphoric acid to a pH of 3.0, and dilute with water to 2000 mL.

Standard solution: 1.0 µg/mL of USP *p*-Chloroaniline RS in *Diluent*

Sample stock solution: Transfer 5.0 mL of Chlorhexidine Gluconate Solution to a 100-mL volumetric flask, and dilute with water to volume.

Sample solution: Transfer 10.0 mL of *Sample stock solution* to a 250-mL volumetric flask, and dilute with *Diluent* to volume.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the amount, in µg/mL, of *p*-chloroaniline in the portion of Chlorhexidine Gluconate Solution taken:

$$\text{Result} = (r_U/r_S) \times D \times C_S$$

r_U = peak response of *p*-chloroaniline from the *Sample solution*

r_S = peak response of *p*-chloroaniline from the *Standard solution*

D = dilution factor used to prepare the *Sample solution*, 500

C_S = concentration of ▲USP *p*-Chloroaniline RS▲ (USP 1-May-2022) in the *Standard solution* (µg/mL)

Acceptance criteria: NMT ▲500 ppm▲ (USP 1-May-2022)

SPECIFIC TESTS

• **Specific Gravity ⟨841⟩:** 1.06–1.07

• **pH ⟨791⟩:** 5.5–7.0, when diluted 1 in 20 with water

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers, protected from light, at controlled room temperature.

Change to read:

• **USP Reference Standards ⟨11⟩**

USP Chlorhexidine RS

USP Chlorhexidine Acetate RS

USP Chlorhexidine System Suitability Mixture RS

This is a mixture containing chlorhexidine and the following impurities (other impurities may also be present):
Chlorhexidine oxazinone analog;

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(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. $C_{13}H_{15}ClN_2O_6$ 330.72

Chlorhexidine amine;
1-(6-Aminohexyl)-5-(4-chlorophenyl)biguanide. $C_{14}H_{23}ClN_6$ 310.83

Chlorhexidine guanidine;
1-[6-(Carbamimidoylamino)hexyl]-5-(4-chlorophenyl)biguanide. $C_{15}H_{25}ClN_8$ 352.87

Chlorhexidine urea;
N-{[6-({[(4-Chlorophenyl)carbamimidoyl]carbamimidoyl}amino)hexyl]carbamimidoyl}urea. $C_{16}H_{26}ClN_9O$ ▲395.90▲ (USP 1-May-2022)

p-Chlorophenyl urea;
1-(4-Chlorophenyl)urea. $C_7H_7ClN_2O$ 170.60

Chlorhexidine nitrile;
1-(4-Chlorophenyl)-5-[6-[(cyanocarbamimidoyl)amino]hexyl]biguanide. $C_{16}H_{24}ClN_9$ 377.88

Chlorhexidine dimer;
1,5-Bis[5-(4-chlorophenyl)biguanidylhexyl]biguanide. $C_{30}H_{47}Cl_2N_{15}$ ▲688.71▲ (USP 1-May-2022)

o-Chlorhexidine;
1-(2-Chlorophenyl)-5-[6-({[(4-chloro phenyl)carbamimidoyl]carbamimidoyl}amino)hexyl]biguanide. $C_{22}H_{30}Cl_2N_{10}$ 505.45

Specified unidentified impurity 2;

Chlorhexidine glucityl triazine;
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. $C_{28}H_{38}Cl_2N_{10}O_5$ ▲665.58▲ (USP 1-May-2022)

Oxochlorhexidine;
N-(4-Chlorophenyl)-*N*′-{[6-({[(4-chlorophenyl)carbamimidoyl]carbamimidoyl}amino)hexyl]carbamimidoyl}urea. $C_{22}H_{29}Cl_2N_9O$ ▲506.44▲ (USP 1-May-2022)

USP *p*-Chloroaniline RS
USP Potassium Gluconate RS

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
CHLORHEXIDINE GLUCONATE SOLUTION	Documentary Standards Support	SM32020 Small Molecules 3

Chromatographic Database Information: Chromatographic Database

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