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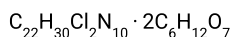
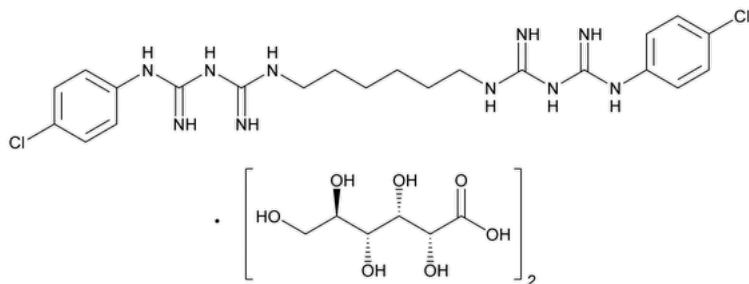
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Chlorhexidine Gluconate Solution

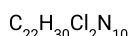
Change to read:



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)

505.45 CAS RN[®]: 55-56-1; UNII: R4K00DY52L.▲ (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 ($\text{C}_{22}\text{H}_{30}\text{Cl}_2\text{N}_{10} \cdot 2\text{C}_6\text{H}_{12}\text{O}_7$).

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**

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)