Chlorhexidine Gluconate Solution

$C_{22}H_{30}Cl_2N_{10} \cdot 2C_6H_{12}O_7$ 897.76
2,4,11,13-Tetraazatetradecanediimidamide, *N*,*N*″-bis(4-chlorophenyl)-3,12-diimino-, di-D-gluconate; 1,1′-Hexamethylenebis[5-(*p*-chlorophenyl)biguanide] di-D-gluconate [18472-51-0].

DEFINITION

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

IDENTIFICATION

- **A. Infrared Absorption** ⟨197K⟩

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

Sample solution: To 1 mL of 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.

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

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

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

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 from the *Sample solution* corresponds in color, size, and R_F value to that from the *Standard solution*.

ASSAY

- **Procedure**

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.

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 and acetonitrile (70:30).

Solution B: Acetonitrile

Mobile phase: See the gradient table below.

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 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 size: 50 µL

System suitability

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

Sample: *System suitability solution*

Suitability requirements

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

Relative standard deviation: NMT 2.0% from the chlorhexidine peak, and NMT 5.0% from the *p*-chloroaniline peak

Analysis

Samples: *Standard solution* and *Sample solution*

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

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

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

r_S = peak area 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, 625.55

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

IMPURITIES

Change to read:

Organic Impurities

- **Procedure 1**

Diluent, Solution A, Solution B, and **Mobile phase:** Proceed as directed in the *Assay*.

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

Sample solution: Transfer 5.0 mL of the *Sample stock solution* to a 25-mL volumetric flask, and dilute with

Diluent to volume. This solution contains 2 mg/mL of chlorhexidine gluconate.

Reference solution A: Transfer 3.0 mL of the *Sample solution* to a 100-mL volumetric flask, and dilute with *Diluent* to volume. This solution contains 0.06 mg/mL of chlorhexidine gluconate.

Reference solution B: Transfer 2.0 mL of *Reference solution A* to a 100-mL volumetric flask, and dilute with *Diluent* to volume. This solution contains 0.0012 mg/mL of chlorhexidine gluconate.

● (RB 1-Apr-2014)

Chromatographic system: Proceed as directed in the *Assay*, except the *Injection size* and chromatograph are programmed as shown in the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	100	0
16	45	55
21	45	55
22	100	0
27	100	0

Injection size: 20 µL

● (RB 1-Apr-2014)

Analysis

Samples: *Sample solution*, *Reference solution A*, and *Reference solution B*

Examine the chromatogram from the *Sample solution*.

Acceptance criteria: The sum of the peak areas, other than chlorhexidine and any peak areas less than that obtained for chlorhexidine from *Reference solution B*, is NMT the peak area for chlorhexidine from *Reference solution A* (3.0%).

- **PROCEDURE 2: LIMIT OF *p*-CHLOROANILINE**

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

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

Sample stock solution: Transfer 5.0 mL of 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. This solution contains 0.4 mg/mL of chlorhexidine gluconate.

Analysis

Samples: *Standard solution* and *Sample solution*

Acceptance criteria: NMT 500 µg/mL

The *p*-chloroaniline peak area response of the *Sample solution* is NMT the *p*-chloroaniline peak area response of the *Standard solution*.

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

● (RB 1-Apr-2014)

USP *p*-Chloroaniline RS
USP Potassium Gluconate RS