- **USP REFERENCE STANDARDS ⟨11⟩**
  USP Chlorhexidine RS
  USP Chlorhexidine Acetate RS
  USP Chlorhexidine Related Compounds RS
  USP *p*-Chloroaniline RS
  USP Potassium Gluconate RS

Chlorhexidine Gluconate Topical Solution

DEFINITION

Chlorhexidine Gluconate Topical Solution is prepared from Chlorhexidine Gluconate Solution. It contains NLT 90.0% and NMT 110.0% of the labeled amount of chlorhexidine gluconate ($C_{22}H_{30}Cl_2N_{10} \cdot 2C_6H_{12}O_7$).

IDENTIFICATION

- **A.** The retention time of the major peak for chlorhexidine from the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST ⟨201⟩**
  Standard solution: 10 mg/mL of USP Potassium Gluconate RS
  Sample solution: Nominally 20 mg/mL of chlorhexidine gluconate from the Topical Solution
  Adsorbent: 0.25-mm layer of chromatographic silica gel
  Application volume: 10 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**
  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. Prepare a mixture of 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 solution: Nominally about 80 µg/mL of chlorhexidine gluconate from the Topical Solution, prepared as follows. Transfer an amount of Topical Solution, equivalent to 40 mg of chlorhexidine gluconate, to a 100-mL volumetric flask, and dilute with methanol to volume. Further dilute a 10-mL portion of this solution with *Solution A* to 50 mL.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: LC
  Detector: UV 239 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L1
  Column temperature: 40°
  Flow rate: 1.5 mL/min
  Injection size: 50 µL
  System suitability
  Sample: *System suitability solution*
  [NOTE—The approximate 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 2.0% for the chlorhexidine peak, NMT 5.0% for the *p*-chloroaniline peak
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of $C_{22}H_{30}Cl_2N_{10} \cdot 2C_6H_{12}O_7$ in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

  r_U = peak area of chlorhexidine from the *Sample solution*
  r_S = peak area of chlorhexidine from the *Standard solution*
  C_S = concentration of USP Chlorhexidine Acetate RS in the *Standard solution* (µg/mL)
  C_U = nominal concentration of chlorhexidine gluconate in the *Sample solution* (µg/mL)
  M_{r1} = molecular weight of chlorhexidine gluconate, 897.76
  M_{r2} = molecular weight of chlorhexidine acetate, 625.55
  Acceptance criteria: 90.0%–110.0%

IMPURITIES

Organic Impurities

- **PROCEDURE: LIMIT OF *p*-CHLOROANILINE**
  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 *Solution A*
  Sample solution: Nominally 0.4 mg/mL of chlorhexidine gluconate from the Topical Solution, prepared as follows. Transfer an amount of Topical Solution, equivalent to 40 mg of chlorhexidine gluconate, to a 100-mL volumetric flask, and dilute with *Solution A* to volume.
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Acceptance criteria
  The *p*-chloroaniline peak area from the *Sample solution* is NMT the *p*-chloroaniline peak area from the *Standard solution* (equivalent to NMT 500 ppm in the portion of Chlorhexidine Gluconate Solution used to prepare the Topical Solution).

SPECIFIC TESTS

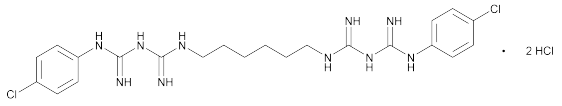
- **pH ⟨791⟩:** 5.0–7.0

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light. Store at controlled room temperature.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Chlorhexidine Acetate RS
USP *p*-Chloroaniline RS
USP Potassium Gluconate RS

Chlorhexidine Hydrochloride



$C_{22}H_{30}Cl_2N_{10} \cdot 2HCl$ 578.37

2,4,11,13-Tetraazatetradecanediimidamide, *N*,*N*″-bis(4-chlorophenyl)-3,12-diimino-, dihydrochloride; 1,1′-Hexamethylenebis[5-(*p*-chlorophenyl)biguanide] dihydrochloride [3697-42-5].

DEFINITION
Chlorhexidine Hydrochloride contains NLT 98.0% and NMT 101.0% of $C_{22}H_{30}Cl_2N_{10} \cdot 2HCl$, calculated on the dried basis.

IDENTIFICATION
• **A. INFRARED ABSORPTION** ⟨197K⟩
Sample: Dissolve 0.3 g in 10 mL of 6 N hydrochloric acid. Add 40 mL of water, filter if necessary, and cool the solution in ice. Add 10 N sodium hydroxide dropwise with stirring until the solution is alkaline to 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°.
Standard: 5 mg/mL USP Chlorhexidine RS in 70% alcohol. Recrystallize this solution, and dry the crystals at 105° for 1 h.
• **B. IDENTIFICATION TESTS—GENERAL,** *Chloride* ⟨191⟩**:** Meets the requirements

ASSAY
• **PROCEDURE**
Solution A: 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. Prepare a mixture of acetonitrile and this solution (3:7).
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

Standard solution: 50 µg/mL of USP Chlorhexidine Acetate RS in *Solution A*
System suitability solution: 50 µg/mL of USP Chlorhexidine Acetate RS and 1 µg/mL of USP *p*-Chloroaniline RS in *Solution A*
Sample solution: 50 µg/mL of Chlorhexidine Hydrochloride in *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
Sample: *System suitability solution*
[NOTE—The approximate relative retention times for chlorhexidine and *p*-chloroaniline are about 1.0 and 1.3, respectively.]
Suitability requirements
Resolution: NLT 3 between chlorhexidine and *p*-chloroaniline
Relative standard deviation: NMT 2.0% for chlorhexidine and NMT 5.0% for *p*-chloroaniline
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of $C_{22}H_{30}Cl_2N_{10} \cdot 2HCl$ in the portion of Chlorhexidine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of chlorhexidine from the *Sample solution*
r_S = peak response of chlorhexidine from the *Standard solution*
C_S = concentration of of USP Chlorhexidine Acetate RS in the *Standard solution* (µg/mL)
C_U = concentration of Chlorhexidine Hydrochloride in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of chlorhexidine hydrochloride, 578.37
M_{r2} = molecular weight of chlorhexidine acetate, 625.55

Acceptance criteria: 98.0%–101.0% on the dried basis

IMPURITIES
Inorganic Impurities
• **RESIDUE ON IGNITION** ⟨281⟩**:** NMT 0.1%
Organic Impurities
• **PROCEDURE**
Solution A and **Solution B:** 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.
Mobile phase: See 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

Sample solution: 2 mg/mL of Chlorhexidine Hydrochloride in *Solution A*
Standard solution A: 0.06 mg/mL of Chlorhexidine Hydrochloride in *Solution A,* from the *Sample solution*
Standard solution B: 1.2 µg/mL of Chlorhexidine Hydrochloride in *Solution A,* from *Standard solution A*
System suitability solution: Transfer 10 mg of USP Chlorhexidine Related Compounds RS to a 10-mL volumetric flask. Dissolve in 2 mL of acetonitrile, and dilute with *Diluent* to volume.
Chromatographic system: Proceed as directed in the *Assay*. [NOTE—Injection size is 20 µL.]
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for the main related compound peak and chlorhexidine are 0.6 and 1.0, respectively.]