pH ⟨791⟩**:** between 5.0 and 7.0.

Limit of *p*-chloroaniline—

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

Standard solutions—Prepare as directed for *Standard solutions* in the test for *Limit of p-chloroaniline* under *Chlorhexidine Gluconate Solution.*

Test solution—Transfer 10.0 mL of Oral Rinse to a 25-mL volumetric flask, dilute with *Diluent* to volume, and mix.

Procedure—Proceed as directed in the test for *Limit of p-chloroaniline* under *Chlorhexidine Gluconate Solution.* Calculate the quantity, in µg per mL, of *p*-chloroaniline in the Oral Rinse taken by the formula:

$$2.5C.$$

The limit is 3.0 µg per mL.

Content of alcohol—

Internal standard solution—Dilute 25 mL of *n*-propyl alcohol with water to 500 mL.

Standard solution—Transfer about 0.25 g of dehydrated alcohol, accurately weighed, to a 28-mL screw capped vial containing about 3 mL of water. Add 5.0 mL of *Internal standard solution,* and dilute with water to almost fill the vial. Cap the vial, and using a vortex mixer, mix for 15 seconds.

Test solution—Transfer about 2.5 g of Oral Rinse, accurately weighed, to a 28-mL screw-capped vial. Add 5.0 mL of *Internal standard solution,* and dilute with water to almost fill the vial. Cap the vial, and using a vortex mixer, mix for 15 seconds.

Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector and a 0.53-mm × 30-m column, the internal wall of which is coated with a 1.5-µm film of liquid phase G27. The column is maintained at about 150° between periods of use. The injection port is equipped with a split injection port with a split ratio of 10:1. The injection port and the detector block temperatures are maintained at about 250° and 275°, respectively. At the time of use the initial column temperature is maintained at about 35° until the alcohol peaks elute, then is increased at a rate of 30° per minute to a final temperature of about 225 °. The carrier gas is helium. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure:* the relative retention times are 1.0 for alcohol and about 1.5 for *n*-propyl alcohol; the resolution, *R,* between alcohol and *n*-propyl alcohol is not less than 2; the tailing factor for the alcohol peak is not more than 3.0; and the relative standard deviation for replicate injections is not more than 2%.

Procedure—Separately inject equal volumes (about 0.5 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the per centage of alcohol (C_2H_5OH) in the Oral Rinse taken by the formula:

$$(W_S / W_U)(R_U / R_S)$$

in which W_S is the weight, in g, of dehydrated alcohol taken to prepare the *Standard solution;* W_U is the weight, in g, of Oral Rinse taken to prepare the *Test solution;* and R_U and R_S are the peak response ratios of alcohol to *n*-propyl alcohol obtained from the *Test solution* and the *Standard solution,* respectively: between 90.0% and 115.0% of the labeled amount of alcohol (C_2H_5OH) is found.

Assay—

Diluent, Solution A, Solution B, Mobile phase, System suitability solution, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Chlorhexidine Gluconate Solution.*

Assay preparation—Transfer 5.0 mL of Oral Rinse to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix.

Procedure—Proceed as directed in the *Assay* under *Chlorhexidine Gluconate Solution.* Calculate the per centage (w/v) of chlorhexidine gluconate ($C_{22}H_{30}Cl_2N_{10} \cdot 2C_6H_{12}O_7$) in the portion of Oral Rinse taken by the formula:

$$(897.76/625.55)(C/500)(r_U / r_S)$$

in which the terms are as defined therein.

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

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.
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*, 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
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.]
Suitability requirements
Resolution: The two peaks between the main related compound peak and the chlorhexidine peak should be at least partially resolved from each other and completely resolved from the chlorhexidine peak.
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 in the chromatogram from *Reference solution B*, is NMT the peak area for chlorhexidine in the chromatogram 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.

- **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.