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# Chlorhexidine Gluconate Topical Gel

## DEFINITION

Chlorhexidine Gluconate Topical Gel is prepared from Chlorhexidine Gluconate Solution. It contains NLT 90.0% and NMT 110.0% of the labeled amount of chlorhexidine gluconate ( $C_{22}H_{30}Cl_2N_{10} \cdot 2C_6H_{12}O_7$ ). [NOTE—The U.S. Food and Drug Administration has not reviewed the safety and efficacy of Chlorhexidine Gluconate Topical Gel and it is not approved for marketing in the United States.]

## IDENTIFICATION

Change to read:

- **A.** ▲The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.▲ (GH 1-Aug-2019)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

Delete the following:

- ▲ **C.** [THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST \(201\)](#).

**Diluent:** [Acetonitrile](#) and water (1:1)

**Standard solution:** 10 mg/mL of [USP Potassium Gluconate RS](#) in *Diluent*

**Sample solution:** Nominally 20 mg/mL of chlorhexidine gluconate from Topical Gel, prepared as follows. Transfer a suitable amount of Topical Gel, equivalent to 500 mg of chlorhexidine gluconate, to a 25-mL volumetric flask. Add a sufficient quantity of *Diluent*, sonicate with intermittent shaking for 30 min, and dilute with *Diluent* to volume. Centrifuge the solution for 5 min at 3000 rpm.

### Chromatographic system

**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 the *Developing 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*.▲ (GH 1-Aug-2019)

## ASSAY

Change to read:

### PROCEDURE

**Buffer:** Dissolve 27.6 g of [sodium dihydrogen 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 2 L.

**Solution A:** [Acetonitrile](#) and *Buffer* (30:70)

**Solution B:** [Acetonitrile](#)

**Mobile phase:** See [Table 1](#).

Table 1

| Time (min) | Solution A (%) | Solution B (%) |
|------------|----------------|----------------|
| 0          | 100            | 0              |
| 9          | 100            |                |

| Time<br>(min) | Solution A<br>(%) | Solution B<br>(%) |
|---------------|-------------------|-------------------|
| 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*

**Sample stock solution:** Nominally 0.4 mg/mL of chlorhexidine gluconate from Topical Gel, prepared as follows. Transfer a suitable amount of Topical Gel, equivalent to 40 mg of chlorhexidine gluconate, to a 100-mL volumetric flask. Add about 70 mL of *Solution A*, sonicate with intermittent shaking for 30 min, and dilute with *Solution A* to volume.

**Sample solution:** Nominally 80 µg/mL of chlorhexidine gluconate from the *Sample stock solution* in *Solution A*

#### Chromatographic system

(See [Chromatography \(621\)](#), [System Suitability](#).)

**Mode:** LC

**Detector:** UV 239 nm. ▲For *Identification A*, use a diode array detector in the range of 200–400 nm. ▲ (GH 1-Aug-2019)

**Column:** 4.6-mm × 25-cm; 5-µm packing [L1](#)

**Column temperature:** 40°

**Flow rate:** 1.5 mL/min

**Injection volume:** 50 µL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of chlorhexidine gluconate ( $C_{22}H_{30}Cl_2N_{10} \cdot 2C_6H_{12}O_7$ ) in the portion of Topical Gel 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 [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

**Change to read:**

##### • LIMIT OF *p*-CHLOROANILINE

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

**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:** 1.0 µg/mL of [USP \*p\*-Chloroaniline RS](#) in *Solution A*

**Sample solution:** Nominally 0.4 mg/mL of chlorhexidine gluconate from Topical Gel, prepared as follows. Transfer a suitable amount of Topical Gel, equivalent to 40 mg of chlorhexidine gluconate, to a 100-mL volumetric flask. Add about 70 mL of *Solution A*, sonicate with intermittent shaking for 30 min, and dilute with *Solution A* to volume. Centrifuge the solution.

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

▲[NOTE—The relative retention times for chlorhexidine and *p*-chloroaniline are 1.0 and 1.3, respectively.]▲ (GH 1-Aug-2019)

#### Suitability requirements

**Resolution:** NLT 3.0 between chlorhexidine and *p*-chloroaniline, *System suitability solution*