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

Add the following:

▲• **C.** The UV spectrum of the major peak of 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. Prepare a mixture of the resulting solution and 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 solution: Nominally about 80 µg/mL of chlorhexidine gluconate from 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. ▲For *Identification C*, use a diode array detector in the range of 200–400 nm.▲ (USP 1-May-2022)

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: *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 2.0% for chlorhexidine; NMT 5.0% for *p*-chloroaniline

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage ▲of the labeled amount of▲ (USP 1-May-2022) chlorhexidine gluconate ($C_{22}H_{30}Cl_2N_{10} \cdot 2C_6H_{12}O_7$) 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, ▲625.56▲ (USP 1-May-2022)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

Change to read:

• **LIMIT OF *p*-CHLOROANILINE**

Solution A, Solution B, Mobile phase, System suitability solution, Chromatographic system, and **▲System suitability:▲** (USP 1-May-2022)

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 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*

▲Calculate the percentage (w/w) of *p*-chloroaniline in the portion of Topical Solution taken:

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

r_U = peak response of *p*-chloroaniline from the *Sample solution*

r_S = peak response of *p*-chloroaniline from the *Standard solution*

C_S = concentration of USP *p*-Chloroaniline RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of chlorhexidine gluconate in the *Sample solution* (mg/mL)

▲ (USP 1-May-2022)

Acceptance criteria: ▲NMT 0.25% (w/w)▲ (USP 1-May-2022)

SPECIFIC TESTS

Add the following:

▲• **Microbial Enumeration Tests ⟨61⟩** and **Tests for Specified Microorganisms ⟨62⟩:** The total aerobic microbial count does not exceed 10^2 cfu/mL. The total yeasts and molds count does not exceed 10^1 cfu/mL. It meets the requirements of the tests for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.▲ (USP 1-May-2022)

• **pH ⟨791⟩:** 5.0–7.0

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers, protected from light. Store at controlled room temperature.

Change to read:

• **USP Reference Standards ⟨11⟩**

USP Chlorhexidine Acetate RS

USP *p*-Chloroaniline RS

▲4-Chloroaniline.

C_6H_6ClN 127.57▲ (USP 1-May-2022)

USP Potassium Gluconate RS

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
CHLORHEXIDINE GLUCONATE TOPICAL SOLUTION	Documentary Standards Support	SM32020 Small Molecules 3

Chromatographic Database Information: Chromatographic Database

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