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Chlorhexidine Gluconate Oral Rinse

DEFINITION

Chlorhexidine Gluconate Oral Rinse 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 of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C.** **Thin-Layer Chromatographic Identification Test** ⟨201⟩

Standard solution: 0.6 mg/mL of USP Potassium Gluconate RS in water

Sample solution: Undiluted Oral Rinse

Chromatographic system

(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 15 µL

Developing solvent system: Alcohol, ethyl acetate, ammonium hydroxide, and water (50:10:10:30)

Spray reagent: Dissolve 2.5 g of ammonium molybdate in about 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*

Separately apply *Standard solution* and the *Sample solution* to a thin-layer chromatographic plate, and allow to dry. Develop the chromatogram in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry at 110° for 20 min. Allow the plate to cool, and spray with *Spray reagent*. Heat the plate at 110° for about 10 min.

Acceptance criteria: The principal spot obtained from the *Sample solution* corresponds in color, size, and R_F 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 *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 60 µg/mL of chlorhexidine gluconate from Oral Rinse, in *Solution A*

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 239 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.

Column: 4.6-mm × 25-cm; base-deactivated 5-µm packing L1

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 0.73% for chlorhexidine; NMT 5.0% for *p*-chloroaniline

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 Oral Rinse 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

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS

• **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 3 mL of water. Add 5.0 mL of *Internal standard solution*, and dilute with water almost filling the vial. Cap the vial, and mix on a vortex mixer for 15 s.

Sample 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 almost filling the vial. Cap the vial, and mix on a vortex mixer for 15 s.

Chromatographic system

(See *Chromatography ⟨621⟩, Gas Chromatography*.)

Mode: GC

Detector: Flame ionization

Column: 0.53-mm × 30-m capillary; coated with a 1.5-µm film of liquid phase G27

Temperatures

Detector: 275°

Injection port: 250°

Column: See *Table 2*.

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Temperature Between Periods of Use (°)
35[a]	30	225	150

[a] Maintain at the initial temperature until the alcohol peaks elute.

Carrier gas: Helium

Injection type: Split, split ratio 10:1

Injection volume: 0.5 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for alcohol and *n*-propyl alcohol are 1.0 and 1.5, respectively.]

Suitability requirements

Resolution: NLT 2 between alcohol and *n*-propyl alcohol

Tailing factor: NMT 3.0 for alcohol

Relative standard deviation: NMT 2% for alcohol

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of alcohol (C_2H_5OH) in the portion of Oral Rinse taken:

$$\text{Result} = (R_U/R_S) \times (W_S/W_U) \times 100$$

R_U = peak response ratio of alcohol to *n*-propyl alcohol from the *Sample solution*

R_S = peak response ratio of alcohol to *n*-propyl alcohol from the *Standard solution*

W_S = weight of dehydrated alcohol taken to prepare the *Standard solution* (g)

W_U = weight of Oral Rinse taken to prepare the *Sample solution* (g)

Acceptance criteria: 90.0%–115.0%

IMPURITIES

Change to read:

• **LIMIT OF *p*-CHLOROANILINE**

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

Diluent: Dissolve 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.

Standard solution: ▲0.001 mg▲ (ERR 1-Dec-2022) /mL of USP *p*-Chloroaniline RS in *Diluent*

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

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage (w/w) of *p*-chloroaniline in the portion of Oral Rinse taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \text{ ▲▲ (ERR 1-Dec-2022)} \times 100$$

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)

▲ ▲ (ERR 1-Dec-2022)

Acceptance criteria: NMT 0.25% (w/w)

SPECIFIC TESTS

• **pH** ⟨791⟩**:** 5.0–7.0

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light, at controlled room temperature.

• **LABELING:** Oral Rinse intended solely for veterinary use is so labeled. Oral Rinse intended for human use is labeled to indicate it is to be expectorated and not swallowed after rinsing.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Chlorhexidine Acetate RS

USP *p*-Chloroaniline RS

4-Chloroaniline.

C_6H_6ClN 127.57

USP Potassium Gluconate RS

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

Topic/Question	Contact	Expert Committee
CHLORHEXIDINE GLUCONATE ORAL RINSE	Documentary Standards Support	SM32020 Small Molecules 3

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:
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