

Status: Currently Official on 14-Feb-2025
Official Date: Official as of 01-Dec-2022
Document Type: USP Monographs
DocId: GUID-D199694B-DF76-4E93-BD74-924BB6F84D3F_6_en-US
DOI: https://doi.org/10.31003/USPNF_M15629_06_01
DOI Ref: 8670b

© 2025 USPC
Do not distribute

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