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Chlorhexidine Acetate Topical Solution

DEFINITION

Chlorhexidine Acetate Topical Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of chlorhexidine acetate ($C_{22}H_{30}Cl_2N_{10} \cdot 2C_2H_4O_2$).

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.** [IDENTIFICATION TESTS—GENERAL \(191\)](#), [Chemical Identification Tests, Acetate](#)
Sample: Evaporate or dilute a volume of Topical Solution containing the equivalent of about 5 mg of chlorhexidine acetate to about 5 mL.
Acceptance criteria: Meets the requirements of test A

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: 40 µg/mL of [USP Chlorhexidine Acetate RS](#) in *Solution A*

Sample solution: Nominally 40 µg/mL of chlorhexidine acetate from the Topical Solution, prepared as follows. Transfer an amount of Topical Solution, equivalent to 20 mg of chlorhexidine acetate, 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 ▲ (USP 1-May-2022) of chlorhexidine acetate ($C_{22}H_{30}Cl_2N_{10} \cdot 2C_2H_4O_2$) in the portion of Topical Solution taken:

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

 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 acetate in the *Sample solution* (µg/mL)**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 2.0 mg/mL of chlorhexidine acetate from the Topical Solution, prepared as follows. Transfer an amount of Topical Solution, equivalent to 200 mg of chlorhexidine acetate, 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 acetate in the *Sample solution* (mg/mL)

▲ (USP 1-May-2022)

Acceptance criteria: ▲NMT 0.05% (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.• **LABELING:** Label it to indicate that it is for veterinary use only.**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)