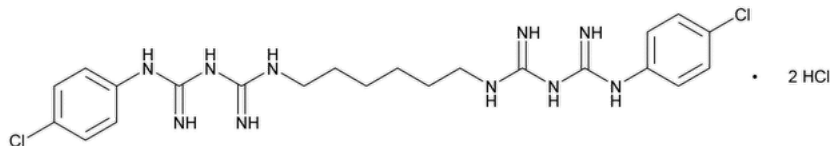


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Chlorhexidine Hydrochloride

Change to read:



$C_{22}H_{30}Cl_2N_{10} \cdot 2HCl$ 578.37

2,4,11,13-Tetraazatetradecanediimidamide, N,N^2 -bis(4-chlorophenyl)-3,12-diimino-, dihydrochloride;
 1,1'-Hexamethylenebis[5-(4-chlorophenyl)biguanide] dihydrochloride CAS RN®: 3697-42-5; UNII: E64XL9U38K.

▲Chlorhexidine (free base)

$C_{22}H_{30}Cl_2N_{10}$ 505.45 CAS RN®: 55-56-1; UNII: R4K00DY52L.▲ (USP 1-May-2022)

DEFINITION

Chlorhexidine Hydrochloride contains NLT 98.0% and NMT 102.0% of chlorhexidine hydrochloride ($C_{22}H_{30}Cl_2N_{10} \cdot 2HCl$), calculated on the dried basis.

IDENTIFICATION

- **A. SPECTROSCOPIC IDENTIFICATION TESTS (197), Infrared Spectroscopy:** 197K

Standard: 5 mg/mL of [USP Chlorhexidine RS](#) in 70% alcohol. Recrystallize this solution, and dry the crystals at 105° for 1 h.

Sample: Dissolve 0.3 g in 10 mL of 6 N hydrochloric acid. Add 40 mL of water, filter if necessary, and cool the solution in ice. Add 10 N sodium hydroxide, dropwise with stirring, until the solution is alkaline to thiazol yellow paper, and add 1 mL in excess. Filter, wash the precipitate with water until the washings are free from alkali, recrystallize the residue from 70% alcohol, and dry the crystals at 105° for 1 h.

Acceptance criteria: Meets the requirements

- **B. IDENTIFICATION TESTS—GENERAL (191), Chemical Identification Tests, Chloride:** Meets the requirements

Add the following:

- **▲C.** The retention time 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: 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 acetonitrile and this solution (3:7).

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	

Time (min)	Solution A (%)	Solution B (%)
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: 50 µg/mL of Chlorhexidine Hydrochloride in *Solution A*

Chromatographic system

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

Mode: LC

Detector: UV 239 nm

Column: 4.6-mm × 25-cm; base-deactivated 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 $\Delta 3.0$ (USP 1-May-2022) between chlorhexidine and p-chloroaniline

Relative standard deviation: NMT 0.73% for chlorhexidine and NMT 5.0% for p-chloroaniline

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of chlorhexidine hydrochloride ($C_{22}H_{30}Cl_2N_{10} \cdot 2HCl$) in the portion of Chlorhexidine Hydrochloride 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 = concentration of Chlorhexidine Hydrochloride in the *Sample solution* (µg/mL)

M_{r1} = molecular weight of chlorhexidine hydrochloride, 578.37

M_{r2} = molecular weight of chlorhexidine acetate, $\Delta 625.56$ (USP 1-May-2022)

Acceptance criteria: 98.0%–102.0% on the dried basis

IMPURITIES

• [RESIDUE ON IGNITION \(281\)](#): NMT 0.1%

• **ORGANIC IMPURITIES**

Store the *System suitability solution*, the *Sample solution*, and the *Diluted sample solution* at a temperature of NMT 12°.

Solution A: 0.1% (v/v) trifluoroacetic acid in acetonitrile

Solution B: 0.1% (v/v) trifluoroacetic acid in water

Solution C: *Solution A* and *Solution B* (20:80)

Solution D: *Solution A* and *Solution B* (90:10)

Mobile phase: See [Table 2](#). Return to original conditions, and equilibrate the system.

Table 2

Time (min)	Solution C (%)	Solution D (%)
0	100	0
2	100	0
32	80	20
37	80	20



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