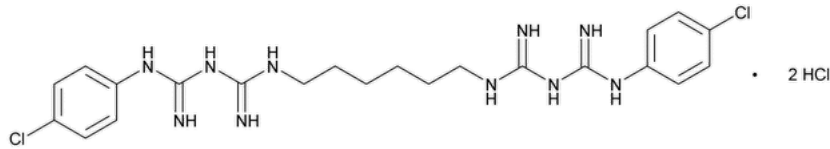


Status: Currently Official on 14-Feb-2025
Official Date: Official as of 01-May-2022
Document Type: USP Monographs
DocId: GUID-933701DC-1DF7-435F-A968-80C401EA550E_3_en-US
DOI: https://doi.org/10.31003/USPNF_M15650_03_01
DOI Ref: vcv0e

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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	0

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

Time (min)	Solution C (%)	Solution D (%)
47	70	30
54	70	30

System suitability solution: 5.0 mg/mL of [USP Chlorhexidine System Suitability Mixture RS](#) in *Solution C*. See [Table 3](#) for the relative retention times of the main components of the mixture.

Table 3

Components of USP Chlorhexidine System Suitability Mixture RS	Relative Retention Time
Chlorhexidine oxazinone analog	0.23
Chlorhexidine amine	0.25
Chlorhexidine guanidine	0.35
Chlorhexidine urea	0.36
<i>p</i> -Chlorophenyl urea	0.5
Chlorhexidine nitrile	0.6
Chlorhexidine dimer	0.85
<i>o</i> -Chlorhexidine	0.90
Specified unidentified impurity 2	0.91
Chlorhexidine glucityl triazine	0.96
Chlorhexidine	1.0
Oxochlorhexidine	1.4

Sample solution: 1.3 mg/mL of Chlorhexidine Hydrochloride in *Solution C*

Diluted sample solution: Dilute 1.0 mL of *Sample solution* with *Solution C* to 100.0 mL.

Chromatographic system

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

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; base-deactivated 5-μm packing [L1](#)

Temperatures

Autosampler: NMT 12°

Column: 30°

Flow rate: 1.0 mL/min

Injection volume: 10 μL

System suitability

Sample: *System suitability solution*

Suitability requirements

Peak-to-valley ratio: NLT 2.0 between chlorhexidine urea and chlorhexidine guanidine

Analysis

Samples: *Sample solution* and *Diluted sample solution*

Calculate the percentage of each impurity in the portion of Chlorhexidine Hydrochloride taken:

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

r_U = peak response of each impurity from the *Sample solution*

r_s = peak response of chlorhexidine from the *Diluted sample solution*

D = dilution factor used to prepare the *Diluted sample solution*, 0.01

Acceptance criteria: See [Table 4](#). The reporting threshold is 0.05%.

Table 4

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Chlorhexidine guanidine	0.35	0.15
Chlorhexidine nitrile	0.6	0.15
Chlorhexidine dimer	0.85	0.2
o-Chlorhexidine and specified unidentified impurity 2	0.90–0.91	0.4 ^a
Chlorhexidine	1.0	—
Oxochlorhexidine	1.4	0.4
Any individual unspecified impurity	—	0.10
Total impurities	—	1.0

^a If present, o-chlorhexidine and specified unidentified impurity 2 may not be completely resolved by the method. These peaks are integrated together to determine conformance.

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.

Standard solution: 1.0 µg/mL of [USP *p*-Chloroaniline RS](#) in *Solution A*

Sample solution: 2.0 mg/mL of Chlorhexidine Hydrochloride in *Solution A*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the amount, in ppm, of *p*-chloroaniline in the portion of Chlorhexidine Hydrochloride taken:

$$\text{Result} = (r_U/r_s) \times (C_s/C_U) \times 0.001 \times 10^6$$

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](#) (USP 1-May-2022) in the *Standard solution* (µg/mL)

C_U = concentration of Chlorhexidine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 500 ppm

SPECIFIC TESTS

• [Loss on Drying \(731\)](#)

Analysis: Dry at 105° to constant weight.

Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.

Change to read:

• [USP REFERENCE STANDARDS \(11\)](#)

[USP Chlorhexidine RS](#)

[USP Chlorhexidine Acetate RS](#)

[USP Chlorhexidine System Suitability Mixture RS](#)

This is a mixture containing chlorhexidine and the following impurities (other impurities may also be present):
Chlorhexidine oxazinone analog;
(5*R*,6*S*)-2-[(4-Chlorophenyl)amino]-5-hydroxy-6-[(1*R*,2*R*)-1,2,3-trihydroxypropyl]-5,6-dihydro-4*H*-1,3-oxazin-4-one.

C₁₃H₁₅ClN₂O₆ 330.72

Chlorhexidine amine;

1-(6-Aminohexyl)-5-(4-chlorophenyl)biguanide. C₁₄H₂₃ClN₆ 310.83

Chlorhexidine guanidine;

1-[6-(Carbamimidoylamino)hexyl]-5-(4-chlorophenyl)biguanide. C₁₅H₂₅ClN₈ 352.87

Chlorhexidine urea;

N-{[6-({[(4-Chlorophenyl)carbamimidoyl] carbamimidoyl)amino]hexyl}carbamimidoyl}urea. C₁₆H₂₆ClN₉O ▲395.90▲ (USP 1-May-2022)

p-Chlorophenyl urea;

1-(4-Chlorophenyl)urea. C₇H₇ClN₂O 170.60

Chlorhexidine nitrile;

1-(4-Chlorophenyl)-5-[6-[(cyanocarbamidoyl)amino]hexyl]biguanide. C₁₆H₂₄ClN₉ 377.88

Chlorhexidine dimer;

1,5-Bis[5-(4-chlorophenyl)biguanidylhexyl]biguanide. C₃₀H₄₇Cl₂N₁₅ ▲688.71▲ (USP 1-May-2022)

o-Chlorhexidine;

1-(2-Chlorophenyl)-5-[6-({[(4-chloro phenyl)carbamimidoyl]carbamimidoyl)amino]hexyl]biguanide. C₂₂H₃₀Cl₂N₁₀ 505.45

Specified unidentified impurity 2;

Chlorhexidine glucityl triazine;

1-(4-Chlorophenyl)-5-[6-({4-[(4-chlorophenyl)amino]-6-[(1*S*,2*R*,3*R*,4*R*)-1,2,3,4,5-pentahydroxypentyl]-1,3,5-triazin-2-yl)amino]hexyl]biguanide. C₂₈H₃₈Cl₂N₁₀O₅ ▲665.58▲ (USP 1-May-2022)

Oxochlorhexidine;

N-(4-Chlorophenyl)-*N*'-[6-({[(4-chloro

phenyl)carbamimidoyl]carbamimidoyl)amino]hexyl]carbamimidoyl}urea. C₂₂H₂₉Cl₂N₉O ▲506.44▲ (USP 1-May-2022)

[USP *p*-Chloroaniline RS](#)

Auxiliary Information - Please [check for your question in the FAQs](#) before contacting USP.

Topic/Question	Contact	Expert Committee
CHLORHEXIDINE HYDROCHLORIDE	Documentary Standards Support	SM32020 Small Molecules 3

Chromatographic Database Information: [Chromatographic Database](#)

Most Recently Appeared In:

Pharmacopeial Forum: Volume No. 46(6)

Current DocID: GUID-933701DC-1DF7-435F-A968-80C401EA550E_3_en-US

DOI: https://doi.org/10.31003/USPNF_M15650_03_01

DOI ref: [vcv0e](#)