Suitability requirements

Resolution: The two peaks between the main related compound peak and the chlorhexidine peak should be at least partially resolved from each other and completely resolved from the chlorhexidine peak.

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

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

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

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

r_S = peak response of chlorhexidine from *Standard solution A*

C_S = concentration of Chlorhexidine Hydrochloride in *Standard solution A* (mg/mL)

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

Acceptance criteria

Total impurities: NMT 3.0%. [NOTE—Disregard any peak less than the area of the chlorhexidine peak as obtained from *Standard solution B*.]

SPECIFIC TESTS

- **LIMIT OF *P*-CHLOROANILINE**

Solution A, Solution B, Mobile phase, System suitability solution, and Chromatographic system: 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*

Acceptance criteria

Total impurities: The *p*-chloroaniline peak area in the chromatogram of the *Sample solution* is NMT the *p*-chloroaniline peak area in the *Standard solution* (NMT 500 ppm).

- **LOSS ON DRYING ⟨731⟩:** Dry a sample at 105 ° to constant weight: it loses NMT 1.0% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
- **USP REFERENCE STANDARDS ⟨11⟩**

USP Chlorhexidine RS
USP Chlorhexidine Acetate RS
USP Chlorhexidine Related Compounds RS
USP *p*-Chloroaniline RS

Chlorophyllin Copper Complex Sodium

DEFINITION

Chlorophyllin Copper Complex Sodium contains sodium salts of copper-chelated chlorophyll derivatives. It contains no artificial coloring.

IDENTIFICATION

- **A. SPECTROPHOTOMETRY AND LIGHT-SCATTERING ⟨851⟩** (in the visible region)

Sample solution: 10 µg/mL

Medium: pH 7.5 phosphate buffer, prepared by mixing 0.15 M dibasic sodium phosphate and 0.15 M monobasic potassium phosphate (21:4)

Acceptance criteria: The ratio of A_{405}/A_{630} is 3.0–3.9.

OTHER COMPONENTS

- **CONTENT OF TOTAL COPPER**

Stock solution 1: 1000 µg/mL of copper. Transfer 1.000 g of copper to a 1000-mL volumetric flask, dissolve in 20 mL of nitric acid, and dilute with 0.2 N nitric acid to volume. [NOTE—Store in a polyethylene bottle.]

Stock solution 2: 10 µg/mL of copper. Transfer 5.0 mL of *Stock solution 1* into a 500-mL volumetric flask, and dilute with water to volume.

Standard solutions: Transfer 5.0, 10.0, 15.0, and 20.0 mL, respectively, of *Stock solution 2* to separate 100-mL volumetric flasks, and dilute the contents of each flask with water to volume. These *Standard solutions* contain 0.5, 1.0, 1.5, and 2.0 µg/mL of copper, respectively.

Sample solution: Transfer 100 mg of previously dried Chlorophyllin Copper Complex Sodium to a Kjeldahl flask. Add 2.0 mL of sulfuric acid, 1.0 mL of nitric acid, and 1.0 mL of hydrogen peroxide, and carefully heat under a fume hood until a light green color is obtained. [NOTE—If the solution has any hint of a brown tint, continue to add 0.5-mL portions of nitric acid until a green color is obtained.] Cool, transfer the contents quantitatively to a 1000-mL volumetric flask with several portions of water, dilute the contents of the flask with water to volume, and mix. Transfer 10.0 mL of this solution to a 50-mL volumetric flask, and dilute with water to volume.

Instrumental conditions

(See *Spectrophotometry and Light-Scattering* ⟨851⟩.)

Mode: Atomic absorption spectrophotometry

Lamp: Copper hollow-cathode

Flame: Air–acetylene

Analytical wavelength: Copper emission line of 324.8 nm

Blank: Water

Analysis

Samples: *Standard solutions* and *Sample solution*

Determine the absorbances of the *Standard solutions* and the *Sample solution*. Plot the absorbances of the *Standard solutions* versus the concentration, in µg/mL, of copper, and draw the straight line best fitting the four plotted points. From the graph so obtained, determine the concentration, *C*, in µg/mL, of copper in the *Sample solution*.

Calculate the percentage of copper in the portion of Chlorophyllin Copper Complex Sodium taken:

$$\text{Result} = (C/W) \times (V/F) \times 100$$

C = concentration of the *Sample solution* determined from the graph (µg/mL)

W = weight of Chlorophyllin Copper Complex Sodium taken to prepare the *Sample solution* (mg)

V = final volume of *Sample solution*, 5000 mL

F = conversion factor, 1000 µg/mg

Acceptance criteria: NLT 4.25% on the dried basis

- **CONTENT OF CHELATED COPPER**

Analysis: Calculate the percentage of chelated copper in the portion of Chlorophyllin Copper Complex Sodium taken by subtracting the percentage of ionic copper found in the test for *Limit of Ionic Copper* from the percentage of total copper found in the test for *Content of Total Copper*.

Acceptance criteria: NLT 4.0% on the dried basis

- **CONTENT OF SODIUM**

Standard stock solution: 100 µg/mL of sodium. Dissolve 254.2 mg of sodium chloride, previously dried at 105 ° for 2 h, in 50 mL of water. Transfer to a 1000-mL volumetric flask, and dilute with water to volume.

Standard solutions: Transfer to each of four 100-mL volumetric flasks 10 mL of a nonionic wetting agent solution (1 in 500). To each flask add, respectively, 2.5, 5.0, 10.0, and 15.0 mL of the *Standard stock solution*, and dilute with water to volume. These *Standard solutions* contain 2.5, 5.0, 10.0, and 15.0 µg/mL of sodium, respectively.

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Blank: Transfer 10 mL of a nonionic wetting agent solution (1 in 500) into a 100-mL volumetric flask, and dilute with water to volume.
Sample solution: Transfer 100 mg of Chlorophyllin Copper Complex Sodium to a 1000-mL volumetric flask. Add 100 mL of a solution of nonionic wetting agent (1 in 500) and 400 mL of water, and shake by mechanical means for 5 min. Dilute with water to volume.
Analysis
Samples: *Standard solutions* and *Sample solution*
Set the flame photometer for maximum transmission at a wavelength of 589 nm. Adjust the instrument to zero transmittance with the *Blank*. Adjust the instrument to 100% transmittance with the most concentrated of the *Standard solutions*. Read the percentage of transmittance of the other *Standard solutions*, and plot the percentage of transmittance versus the concentration, in µg/mL, of sodium. Read the percentage of transmittance of the *Sample solution*, and from the graph read the concentration, *C*, in µg/mL, of sodium in the *Sample solution*.
Calculate the percentage of sodium in the portion of Chlorophyllin Copper Complex Sodium taken:

$$\text{Result} = (C/W) \times (V/F) \times 100$$

C = concentration of the *Sample solution* determined from the graph (µg/mL)
W = weight of Chlorophyllin Copper Complex Sodium taken to prepare the *Sample solution* (mg)
V = volume of *Sample solution*, 1000 mL
F = conversion factor, 1000 µg/mg
Acceptance criteria: 5%–7% on the dried basis
- **NITROGEN DETERMINATION,** *Method I* ⟨**461**⟩**:** NLT 4.0%

IMPURITIES
- **LIMIT OF IONIC COPPER**
Standard solutions: Prepare as directed in the test for *Content of Total Copper.*
Sample solution: Transfer 100 mg of Chlorophyllin Copper Complex Sodium to a 150-mL conical flask. Add 75 mL of water, and shake by mechanical means for 3 min. Adjust with 1 N hydrochloric acid to a pH of 3.0, transfer the suspension thus obtained to a 100-mL volumetric flask, and dilute with water to volume. Filter this suspension, discarding the first 10 mL of the filtrate. Use the clear filtrate for analysis.
Analysis
Samples: *Standard solutions* and *Sample solution*
Proceed as directed in the test for *Content of Total Copper*.
Calculate the percentage of ionic copper in the portion of Chlorophyllin Copper Complex Sodium taken:

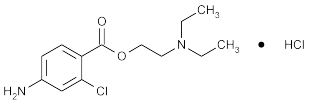
$$\text{Result} = (C/W) \times (V/F) \times 100$$

C = concentration of the *Sample solution* determined from the graph (µg/mL)
W = weight of Chlorophyllin Copper Complex Sodium taken to prepare the *Sample solution* (in mg on the dried basis)
V = volume of *Sample solution*, 100 mL
F = conversion factor, 1000 µg/mg
Acceptance criteria: NMT 0.25% on the dried basis
- **RESIDUE ON IGNITION** ⟨**281**⟩**:** NMT 30% on the dried basis
- **ARSENIC,** *Method II* ⟨**211**⟩**:** NMT 3 ppm
- **LEAD** ⟨**251**⟩**:** NMT 10 ppm
- **IRON** ⟨**241**⟩**:** NMT 0.50%

SPECIFIC TESTS
- **MICROBIAL ENUMERATION TESTS** ⟨**61**⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨**62**⟩**:** It meets the requirements of the tests for absence of *Escherichia coli* and *Salmonella* species.
- **PH** ⟨**791**⟩**:** 9.5–10.7, in a solution (1 in 100)
- **LOSS ON DRYING** ⟨**731**⟩**:** Dry a sample at 150 ° for 2 h: it loses NMT 5% of its weight.
- **TEST FOR FLUORESCENCE**
Sample solution: 10 mg/mL
Analysis: Apply 10 µL of *Sample solution* on filter paper, allow to dry, and examine the area of application under long-wavelength UV light through a red optical filter.
Acceptance criteria: No fluorescence is visible.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

Chloroprocaine Hydrochloride



$C_{13}H_{19}ClN_2O_2 \cdot HCl$ 307.22
Benzoic acid, 4-amino-2-chloro-, 2-(diethylamino)ethyl ester, monohydrochloride.
2-(Diethylamino)ethyl 4-amino-2-chlorobenzoate monohydrochloride [3858-89-7].

» Chloroprocaine Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of $C_{13}H_{19}ClN_2O_2 \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩**—**
USP Chloroprocaine Hydrochloride RS

Identification—
A: *Infrared Absorption* ⟨197K⟩.
B: *Ultraviolet Absorption* ⟨197U⟩—
Solution: 10 µg per mL.
Medium: pH 4.5 buffer solution [prepared by dissolving 13.61 g of monobasic potassium phosphate in 750 mL of water, adjusting to a pH of 4.5 ± 0.1 with potassium hydroxide solution (1 in 180) and diluting with water to 1000 mL]. Absorptivities at 290 nm, calculated on the dried basis, do not differ by more than 3.0%.
C: It meets the requirements of the tests for *Chloride* ⟨191⟩.

Melting range, *Class I* ⟨741⟩: between 173 ° and 176 °.

Acidity—Dissolve 1.0 g in 25 mL of water, add 2 drops of methyl red TS, and titrate with 0.020 N sodium hydroxide: not more than 1.8 mL is required to produce a yellow color.

Loss on drying ⟨731⟩**—**Dry about 500 mg, accurately weighed, at 105 ° for 2 hours: it loses not more than 1.0% of its weight.

Residue on ignition ⟨281⟩: not more than 0.2%.

Related compounds—
4-Amino-2-chlorobenzoic acid—Using the chromatograms obtained as directed for the *Assay*, calculate the percentage of 4-amino-2-chlorobenzoic acid ($C_7H_6ClNO_2$) in the Chloroprocaine Hydrochloride taken by the formula:

$$10{,}000(C / W)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of 4-amino-2-chlorobenzoic acid in the *Standard preparation; W* is the quantity, in mg, of Chloroprocaine Hydrochloride taken to prepare the *Assay preparation*; and r_U and r_S are the 4-amino-2-chlorobenzoic acid peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively: not more than 0.625% is found.

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