

lent to 0.050 g of Chlordiazepoxide according to the labeled amount, add exactly 5 mL of a mixture of methanol and ammonia TS (97:3), shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 0.050 g of Chlordiazepoxide Reference Standard in a mixture of methanol and ammonia TS (97:3) to make exactly 50 mL, and use this solution as the standard solution (1). Dissolve 5.0 mg of 2-amino-5-chlorobenzophenone for thin-layer chromatography in methanol to make exactly 200 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 25 μ L of the sample solution and 10 μ L each of the standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Proceed as directed in the Purity (2) under Chlordiazepoxide.

Dissolution test Perform the test with 1 tablet of Chlordiazepoxide Tablets at 100 revolutions per minute according to Method 2 under the Dissolution Test, using 900 mL of diluted phosphate buffer solution, pH 6.8, (1 in 2) as the test solution. Take 30 mL or more of the dissolved solution 60 minutes after start of the test, and filter through a membrane filter with pore size of not more than 0.8 μ m. Discard the first 10 mL of the filtrate, pipet the subsequent V mL, add diluted phosphate buffer solution, pH 6.8, (1 in 2) to make exactly V' mL so that each mL contains about 3.7 μ g of chlordiazepoxide ($C_{16}H_{14}ClN_3O$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 0.012 g of chlordiazepoxide for assay, previously dried in a desiccator for 4 hours (in vacuum, phosphorus (V) oxide, 60°C), and dissolve in diluted phosphate buffer solution, pH 6.8, (1 in 2) to make exactly 200 mL. Pipet 3 mL of this solution, add diluted phosphate buffer solution, pH 6.8, (1 in 2) to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and the standard solution at 260 nm as directed under the Ultraviolet-visible Spectrophotometry.

The dissolution rate of Chlordiazepoxide Tablets in 60 minutes is not less than 70%.

Dissolution rate (%) with respect to the labeled amount of chlordiazepoxide ($C_{16}H_{14}ClN_3O$)

$$= W_S \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times 27$$

W_S : Amount (mg) of chlordiazepoxide for assay.

C : Labeled amount (mg) of chlordiazepoxide ($C_{16}H_{14}ClN_3O$) in 1 tablet.

Assay Conduct this procedure without exposure to daylight, using light-resistant vessels. Weigh accurately a quantity of powdered Chlordiazepoxide Tablets, equivalent to about 0.1 g of Chlordiazepoxide ($C_{16}H_{14}ClN_3O$), transfer to a glass-stoppered flask, add 10 mL of water, and shake well to disintegrate. Add 60 mL of methanol, shake well, add methanol to make exactly 100 mL, and centrifuge. Pipet 10 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, add methanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.01 g of Chlordiazepoxide Reference Standard, previously dried in a desiccator (in vacuum, phosphorus (V) oxide, 60°C) for 4 hours, dissolve in 1 mL of water and methanol, add exactly 5 mL of the internal

standard solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μ L each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following operating conditions, and calculate the ratios, Q_T and Q_S , of the peak area of chlordiazepoxide to that of the internal standard.

$$\begin{aligned} &\text{Amount (mg) of chlordiazepoxide (C}_{16}\text{H}_{14}\text{ClN}_3\text{O)} \\ &= \text{amount (mg) of Chlordiazepoxide} \\ &\quad \text{Reference Standard} \\ &\quad \times \frac{Q_T}{Q_S} \times 10 \end{aligned}$$

Internal standard solution—A solution of isobutyl salicylate in methanol (1 in 20).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4 mm in inside diameter and 25 to 30 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (10 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and 0.02 mol/L ammonium dihydrogenphosphate TS (7:3).

Flow rate: Adjust the flow rate so that the retention time of chlordiazepoxide is about 5 minutes.

Selection of column: Proceed with 10 μ L of the standard solution under the above operating conditions. Use a column giving elution of chlordiazepoxide and the internal standard in this order with the resolution between these peaks being not less than 9.

Containers and storage Containers—Tight containers.

Chlorhexidine Gluconate Solution

ゲルコン酸クロルヘキシジン液

Chlorhexidine Gluconate Solution is a solution of digluconate of chlorhexidine.

It contains not less than 19.0 w/v% and not more than 21.0 w/v% of chlorhexidine gluconate ($C_{22}H_{30}Cl_2N_{10} \cdot 2C_6H_{12}O_7$; 897.76).

Description Chlorhexidine Gluconate Solution is a clear, colorless or pale yellow liquid. It is odorless, and has a bitter taste.

It is miscible with water and with acetic acid (100). 1 mL of Chlorhexidine Gluconate Solution is miscible with not more than 5 mL of ethanol (99.5) and with not more than 3 mL of acetone. By further addition of each of these solvents, a white turbidity is formed.

It is gradually colored by light.

Specific gravity d_{20}^{20} : 1.06 – 1.07

Identification (1) To 0.05 mL of Chlorhexidine Gluconate Solution add 5 mL of methanol, 1 mL of bromine TS and 1 mL of 8 mol/L sodium hydroxide TS: a deep red color is produced.

(2) To 0.5 mL of Chlorhexidine Gluconate Solution add

10 mL of water and 0.5 mL of copper (II) sulfate TS: a white precipitate is formed. Heat to boiling: the precipitate changes to light purple.

(3) To 10 mL of Chlorhexidine Gluconate Solution add 5 mL of water, cool on ice, and add 5 mL of sodium hydroxide TS dropwise with stirring: a white precipitate is formed. Collect the precipitate by filtration, wash with water, recrystallize from diluted ethanol (95) (7 in 10), and dry at 105°C for 30 minutes: the crystals thus obtained melt between 130°C and 134°C.

(4) Neutralize the filtrate obtained in (3) with 5 mol/L hydrochloric acid TS. To 5 mL of this solution add 0.65 mL of acetic acid (100) and 1 mL of freshly distilled phenylhydrazine, and heat on a water bath for 30 minutes. After cooling, scratch the inner wall of the vessel with a glass rod to induce crystallization. Collect the crystals, dissolve in 10 mL of hot water, add a small amount of activated charcoal, and filter. Cool the filtrate, scratch the inner side of the vessel, collect the formed crystals, and dry: the crystals thus obtained melt at about 195°C (with decomposition).

pH To 5.0 mL of Chlorhexidine Gluconate Solution add water to make 100 mL: the pH of the solution is between 5.5 and 7.0.

Purity *p*-Chloroaniline—To 2.0 mL of Chlorhexidine Gluconate Solution add water to make exactly 100 mL. Pipet 5 mL of the solution, and add 20 mL of water and 5 mL of 1 mol/L hydrochloric acid TS. Add 0.3 mL of sodium nitrite TS, shake, and allow to stand for 2 minutes. Add 4 mL of ammonium amidosulfate TS, and then allow to stand for 1 minute. Add 5 mL of *N*-(1-naphthyl)-*N'*-diethylethylenediamine oxalate-acetone TS, allow to stand for 10 minutes, add 1 mL of ethanol (95), and then add water to make 50 mL: the color of the solution is not more intense than the following control solution.

Control solution: Dissolve 0.020 g of 4-chloroaniline in 10 mL of 1 mol/L hydrochloric acid TS, and add water to make exactly 100 mL. Pipet 5 mL of the solution, and add water to make exactly 100 mL. Pipet 5 mL of the solution, add 20 mL of water and 5 mL of 1 mol/L hydrochloric acid TS, and proceed as directed for the preparation of the sample solution.

Residue on ignition Not more than 0.10% (2 g, after evaporation).

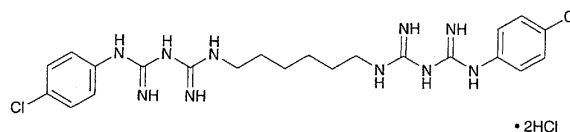
Assay Pipet 2 mL of Chlorhexidine Gluconate Solution, evaporate to dryness on a water bath, dissolve the residue in 60 mL of acetic acid for nonaqueous titration, and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 22.444 mg of $C_{22}H_{30}Cl_2N_{10} \cdot 2C_6H_{12}O_7$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Chlorhexidine Hydrochloride

塩酸クロルヘキシジン



$C_{22}H_{30}Cl_2N_{10} \cdot 2HCl$: 578.37
1,1'-Hexamethylenebis[5-(4-chlorophenyl)biguanide] dihydrochloride [3697-42-5]

Chlorhexidine Hydrochloride, when dried, contains not less than 98.0% of $C_{22}H_{30}Cl_2N_{10} \cdot 2HCl$.

Description Chlorhexidine Hydrochloride occurs as a white, crystalline powder. It is odorless, and has a bitter taste.

It is soluble in formic acid, slightly soluble in methanol and in warm methanol, and practically insoluble in water, in ethanol (95) and in diethyl ether.

It is gradually colored by light.

Identification (1) Dissolve 0.01 g of Chlorhexidine Hydrochloride in 5 mL of methanol by warming, and add 1 mL of bromine TS and 1 mL of 8 mol/L sodium hydroxide TS: a deep red color is produced.

(2) Dissolve 0.3 g of Chlorhexidine Hydrochloride in 10 mL of 6 mol/L hydrochloric acid TS, cool in ice, and add 10 mL of 8 mol/L sodium hydroxide TS dropwise with stirring: a white precipitate is produced. Collect the precipitate, wash with water, recrystallize from diluted ethanol (95) (7 in 10), and dry at 105°C for 30 minutes: the crystals so obtained melt between 130°C and 134°C.

(3) Dissolve 0.1 g of Chlorhexidine Hydrochloride in 50 mL of dilute nitric acid: the solution responds to the Qualitative Tests for chloride.

Purity (1) Heavy metals—Proceed with 2.0 g of Chlorhexidine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic—To 1.0 g of Chlorhexidine Hydrochloride in a crucible add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), fire the ethanol (95) to burn, and heat gradually to incinerate. If a carbonized substance remains, moisten with a small amount of nitric acid, and ignite to incinerate. Cool, add 10 mL of dilute hydrochloric acid to the residue, dissolve by warming on a water bath, use this solution as the test solution, and perform the test using Apparatus B (not more than 2 ppm).

(3) *p*-Chloroaniline—Dissolve 0.10 g of Chlorhexidine Hydrochloride in 2 mL of formic acid, and add 15 mL of 1 mol/L hydrochloric acid TS and 20 mL of water immediately. Add 0.3 mL of sodium nitrite TS, shake, and allow to stand for 2 minutes. Add 4 mL of ammonium amidosulfate TS, and then allow to stand for 1 minute. Add 5 mL of *N*-(1-naphthyl)-*N'*-diethylethylenediamine oxalate-acetone TS, allow to stand for 10 minutes, and add 1 mL of ethanol (95) and water to make 50 mL: the solution has no more color than the following control solution.

Control solution: Dissolve 0.020 g of 4-chloroaniline in