DRAFT PROPOSAL FOR THE INTERNATIONAL PHARMACOPOEIA:

CHLORHEXIDINE DIGLUCONATE TOPICAL SOLUTION
(CHLORHEXIDINI DIGLUCONATIS SOLUTIO TOPICALUM)
(June 2016)

DRAFT FOR COMMENT

Should you have any comments on the attached text, please send these to Dr Herbert Schmidt, Medicines Quality Assurance, Technologies, Standards and Norms, World Health Organization, 1211 Geneva 27, Switzerland; email: schmidt@who.int; fax: (+41 22) 791 4730 by 12 August 2016.

In order to speed up the process for receiving draft monographs and for sending comments, please let us have your email address (to bonnyw@who.int) and we will add it to our electronic mailing list. Please specify if you wish to receive monographs.

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Please send any request for permission to:

Dr Sabine Kopp, Group Lead, Medicines Quality Assurance, Technologies, Standards and Norms, Department of Essential Medicines and Health Products, World Health Organization, CH-1211 Geneva 27, Switzerland.
Fax: (41-22) 791 4730; email: kopp@who.int.

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Draft proposal for *The International Pharmacopoeia:*

**CHLORHEXIDINE DIGLUCONATE TOPICAL SOLUTION**

*(CHLORHEXIDINI DIGLUCONATIS SOLUTIO TOPICALUM)*

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<tr>
<td>First draft received from collaborating laboratory</td>
<td>September 2015</td>
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<tr>
<td>Presentation to WHO Expert Committee on Specifications for Pharmaceutical Preparations for information and discussion</td>
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<td>Draft revision sent out for public consultation</td>
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**Chlorhexidine digluconate topical solution**  
*(Chlorhexidini digluconatis solutio topicalum)*

**Category.** Antiseptic.

**Storage.** Chlorhexidine digluconate topical solution should be kept in a well-closed container, protected from light.

**Additional information.** Strength in the current WHO Model List of Essential Medicines (EML): 5% (digluconate) and 7.1% (digluconate), delivering 4% chlorhexidine (for umbilical cord care); strengths in the current EML for children: 5% (digluconate) and 7.1% (digluconate), delivering 4% chlorhexidine (for umbilical cord care).

**Definition.** Chlorhexidine digluconate topical solution is a solution of Chlorhexidine digluconate solution in a suitable vehicle. It contains not less than 90.0% and not more than 110.0% of the amount of chlorhexidine digluconate \((C_{22}H_{30}Cl_{2}N_{10}\cdot2C_{6}H_{12}O_{7})\) stated on the label.

**Identity tests**

**A.** Carry out the test as described under *1.14.1 Thin-layer chromatography* using silica gel R5 as the coating substance and a mixture of 50 volumes of dehydrated ethanol R, 10 volumes of ethyl acetate R, 10 volumes of ammonia \((\sim260 \text{ g/L})\) TS R and 30 volumes of water R as the mobile phase. Apply separately to the plate 2 μL of each of the following 2 solutions in water R. For solution (A) dilute a quantity of the topical solution to obtain a solution containing 20 mg of chlorhexidine digluconate per mL. For solution (B) use a solution containing 10 mg of potassium gluconate R per mL. After removing the plate from the chromatographic chamber heat the plate for 20 minutes at 110 °C and allow the plate to cool. Spray with ammonium molybdate/ceric sulfate/sulfuric acid TS. Heat the plate for 10 minutes at 110 °C. Examine the chromatogram in daylight.

The principal spot obtained with solution (A) corresponds in position, appearance and intensity to that obtained with solution (B).

**B.** Transfer a quantity of the topical solution containing 5 mg of chlorhexidine digluconate into a 500 mL volumetric flask and dilute to volume with water R. The absorption spectrum \((1.6)\) of the resulting solution, when observed between 200 nm and 320 nm, exhibits two maxima at about 231 nm and 255 nm, and two minima at about 218 nm and 242 nm.

**C.** Carry out the test as described under *1.14.4 High-performance liquid chromatography* using the conditions given under “Assay”. The retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to the retention time of the peak due to chlorhexidine in the chromatogram obtained with solution (2).

**pH value \((1.13)\).** 5.0–7.0.

**Impurity P (4-Chloroaniline)**

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*Draft for comment*
Prepare fresh solutions and perform the tests without delay. Carry out the test as described under 1.14.4 High-performance liquid chromatography using the same chromatographic conditions as described under “Assay”.

Prepare the following solutions in mobile phase A:

For solution (1) transfer an amount of the topical solution, equivalent to 40.0 mg chlorhexidine digluconate to a 100 mL volumetric flask, and dilute to volume.

For solution (2) use 1.0 μg of 4-chloroaniline R per mL.

For solution (3) prepare a solution that contains 50 μg per mL of chlorhexidine diacetate RS and 1 μg per mL 4-chloroaniline R.

Inject 50 μL of solution (3) In the chromatogram obtained with solution (3) the peak due to 4-chloroaniline is eluted at a relative retention of about 1.3 with reference to chlorhexidine (retention time about 6 minutes). The test is not valid unless the resolution between the peaks due to 4-chloroaniline and chlorhexidine is at least 3.0.

Inject alternately 50 μL of each of solutions (1) and (2).

Measure the areas of the peaks corresponding to 4-chloroaniline obtained in the chromatograms of solutions (1) and (2).

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to 4-chloroaniline is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.05% (m/v) with reference to the concentration of chlorhexidine digluconate in the topical solution).

Assay

Prepare fresh solutions and perform the tests without delay.

Carry out the test as described under 1.14.4 High-performance liquid chromatography using a stainless steel column (25 cm x 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilane groups (5 μm).¹

Use the following conditions for gradient elution:

mobile phase A: Dissolve 27.6 g of sodium dihydrogen phosphate R and 10 mL of triethylamine R in 1.5 L water. Adjust the pH to 3.0 using phosphoric acid (~1440 g/L) TS. Dilute with water R to a final volume of 2 L. Prepare a 70:30 mixture of the resulting solution and acetonitrile R;

mobile phase B: Acetonitrile R.

¹ Symmetry C18, 250 mm x 4.6 mm – 5 μm was found suitable.
Operate with a flow rate of 1.5 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 239 nm.

Maintain the column temperature at 40°C.

Prepare the following solutions:

For solution (1) transfer an amount of the topical solution, equivalent to about 40 mg chlorhexidine digluconate, accurately weighed, to a 100 mL volumetric flask, and dilute to volume with methanol R. Further dilute a 10 mL portion of this solution with mobile phase A to 50 mL.

For solution (2) use 50 μg of chlorhexidine diacetate RS per mL in mobile phase A.

For solution (3) prepare a solution that contains 50 μg per mL of chlorhexidine diacetate RS and 1 μg per mL 4-chloroaniline R in mobile phase A.

Inject 50 μL of solution (3).

In the chromatogram obtained with solution (3) the peak due to 4-chloroaniline is eluted at a relative retention of about 1.3 with reference to chlorhexidine (retention time about 6 minutes). The assay is not valid unless the resolution between the peaks due to 4-chloroaniline and chlorhexidine is at least 3.0.

Inject alternately 50 μL of each of solutions (1) and (2).

Measure the areas of the peaks corresponding to chlorhexidine obtained in the chromatograms of solutions (1) and (2). Determine the weight per mL (1.3.1) and calculate the percentage content of chlorhexidine digluconate, weight in volume, in the topical solution using the declared content of chlorhexidine in chlorhexidine diacetate RS (C_{22}H_{30}Cl_{2}N_{10}·2C_{6}H_{12}O_{7}). Each mg of chlorhexidine (C_{22}H_{30}Cl_{2}N_{10}) is equivalent to 1.776 mg of chlorhexidine digluconate (C_{22}H_{30}Cl_{2}N_{10}·2C_{6}H_{12}O_{7}).

Impurities. The impurity limited by the requirements of this monograph is impurity P listed in the monograph for Chlorhexidine digluconate solution.
Reagents to be added to Reagents, test solutions and volumetric solutions

Potassium gluconate R

\[ \text{C}_6\text{H}_{11}\text{KO}_7 \]

Reference substances to be established

Chlorhexidine diacetate RS

4-chloroaniline RS

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