Nonoxinol 9 (Nonoxinolum 9)

C₉H₁₉C₆H₄(OCH₂CH₂)nOH

(Average value of n = 9, with a possible range of 4-16.)

**Chemical name.** Polyethylene glycol mono(\(p\)-nonylphenyl) ether; \(\alpha\)-\((4\)-nonylphenyl\)-\(\omega\)-hydroxypoly-(oxy-1,2-ethanediyl); CAS Reg. No. 26027-38-3.

**Description.** A clear, colourless to light yellow, viscous liquid.

**Solubility.** Miscible with water, ethanol (~750 g/l) TS and olive oil R.

**Category.** Adjunctive contraceptive agent.

**Storage.** Nonoxinol 9 should be kept in a tightly closed container.

**Additional Information.** Nonoxinol 9 should be kept away from oxidizing agents.

**Requirements**

**Definition.** Nonoxinol 9 is an anhydrous liquid mixture containing mainly monononylphenyl ethers of macrogols.

The content is not less than 95.0% and not more than 105.0% of Nonoxinol 9, calculated with reference to the anhydrous substance.

**Identity tests**

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the reference spectrum of nonoxinol 9.

B. See the test described below under "Assay". The retention time of the major peak in the chromatogram obtained with solution A corresponds to that in the chromatogram obtained with solution B.

**Acid value.** Not more than 0.2.

**Water.** Determine as described under 2.8 Determination of water by the Karl Fischer method. Method A, using about 0.5 g of the substance; the water content is not more than 5.0 mg/g.

**Macrogol.** Transfer about 10 g, accurately weighed, to a 250-mL beaker. Add 100 mL of ethyl acetate R and allow to dissolve using a magnetic stirrer. Transfer to a 500-mL separatory funnel fitted with a glass stopper with the aid of 100 mL of sodium chloride (300 g/l) TS. Insert the stopper and shake vigorously for 1 minute. Remove the stopper carefully. Immerse a thermometer into the mixture and place the funnel so that it is partially immersed in a water-bath maintained at 50 °C. Swirl the funnel gently while letting the internal temperature rise to between 40 and 45 °C. Once this is reached remove the funnel from the bath immediately, dry the outside surface, and drain the aqueous layer into another 500-mL separatory funnel. Discard the organic layer. Wash the combined aqueous layers with 100 mL of ethyl acetate R and separate the aqueous layer into another 500-mL separatory funnel. Discard the organic layer. Extract the aqueous layer with two successive 100-mL portions of dichloromethane R, filtering the organic layers through a folded filter paper (e.g. grade Whatman 2V) and combining them in a 250-mL beaker. Evaporate to dryness on a water-bath and continue heating until the odour of dichloromethane is no longer perceptible. Allow the beaker to cool. Add 25 mL of acetone R and allow the residue to dissolve using a magnetic stirrer. Filter into a tared 250-mL beaker, rinsing with two 25-mL quantities of acetone R. Evaporate to dryness on a water-bath. Dry under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) at 60 °C for 1 hour. Allow the beaker to cool, and weigh; not more than 16 mg/g.

**Cloudiness of solution.** Transfer 1.0 g to a 250-mL beaker, add 99 g of water, and mix to dissolve. Pour about 30 mL of the solution into a 70-mL test-tube. Place the test-tube into a water-bath and stir the contents constantly with a thermometer until the solution becomes cloudy, then immediately remove the test-tube from the bath, so that the temperature does not rise further by more than 2°C, and continue stirring. The temperature at which the solution becomes sufficiently clear and when the entire thermometer bulb is clearly visible is between 52 and 56 °C.

**Ethylene oxide and dioxan.** Carry out the test as described under 1.14.5 Gas chromatography, with the apparatus equipped with an injection system for the performance of head-space chromatography. Use a capillary glass or quartz column (30m × 0.32 mm), the inner surface of which is coated with a thick layer of polydimethylsiloxane R (1.0 μm). Maintain the temperature of the
column at 50 °C for 5 minutes. Increase the temperature at a rate of 5°C per minute to 180 °C, and then increase the temperature again at a rate of 30°C per minute to 230 ºC, and maintain it at this point for 5 minutes. Maintain the temperature of the injection port at 150 °C and that of the detector at 250 °C. Use helium R or nitrogen R as the carrier gas with a linear velocity of about 20 cm per second and a split ratio of 1: 20; use a flame-ionization detector.

Prepare the following solutions: for solution (A) weigh 1.0 g of Nonoxinol 9, add 1.0 mL of water, mix to obtain a homogeneous solution, and allow to stand at 70 °C for 45 minutes; for solution (B) weigh 1.0 g of Nonoxinol 9, add 0.5 mL of ethylene oxide TS and 0.5 mL of dioxan TS, mix to obtain a homogeneous solution, and allow to stand at 70 ºC for 45 minutes; for solution (C) add to 0.5 mL of ethylene oxide TS 0.10 mL of a freshly prepared 10 mg/l solution of acetaldehyde R and 0.10 mL of dioxan TS, mix to obtain a homogeneous solution, and allow to stand at 70 °C for 45 minutes.

Inject 1.0 mL of the gaseous phase of solution C. Adjust the sensitivity of the system so that the heights of the peaks corresponding to ethylene oxide and acetaldehyde in the chromatogram obtained are at least 15% of the full scale of the recorder. The test is not valid unless the resolution between the peaks corresponding to acetaldehyde and ethylene oxide is at least 2.0 and the peak of ethylene oxide is detected with a signal-to-noise ratio of at least 5.

Inject separately 1.0 mL each of the gaseous phases of solutions A and B.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and B. The mean areas of the ethylene oxide and dioxan peaks obtained with solution A are not greater than half the mean area of the corresponding peak obtained with solution B (1mg/g of ethylene oxide and 50mg/g of dioxan).

**Assay.** Determine by 1.14.4 High-performance liquid chromatography, using a stainless steel column (25 cm x 4 mm) packed with particles of porous silica, the surface of which has been modified with chemically bonded dihydroxypropane groups (dial) (10 μm). As mobile phase A, use a mixture of 2 volumes of ethyl acetate R and 8 volumes of hexane R. As mobile phase B, use a mixture of 2.5 volumes of methanol R and 97.5 volumes of ethyl acetate R.

Prepare the following solutions in mobile phase A: solution (A) 2.0 mg of Nonoxinol 9 per mL; and solution (B) 2.0 mg of nonoxinol 9 RS per mL.

Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 280 nm.

Use the following gradient elution system:

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Mobile phase A (% v/v)</th>
<th>Mobile phase B (% v/v)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-2</td>
<td>100</td>
<td>0</td>
<td>equilibration</td>
</tr>
<tr>
<td>2-10</td>
<td>100 → 84</td>
<td>0 → 16</td>
<td>linear gradient</td>
</tr>
<tr>
<td>10-20</td>
<td>84 → 70</td>
<td>16 → 30</td>
<td>linear gradient</td>
</tr>
<tr>
<td>20-30</td>
<td>70 → 62</td>
<td>30 → 38</td>
<td>linear gradient</td>
</tr>
<tr>
<td>30-40</td>
<td>62 → 57</td>
<td>38 → 43</td>
<td>linear gradient</td>
</tr>
<tr>
<td>40-50</td>
<td>57 → 54</td>
<td>43 → 46</td>
<td>linear gradient</td>
</tr>
<tr>
<td>50-70</td>
<td>54 → 50</td>
<td>46 → 50</td>
<td>linear gradient</td>
</tr>
<tr>
<td>70-75</td>
<td>50 → 50</td>
<td>50 → 50</td>
<td>isocratic</td>
</tr>
<tr>
<td>75-76</td>
<td>50 → 100</td>
<td>50 → 0</td>
<td>re-equilibration</td>
</tr>
</tbody>
</table>

Inject 100 μl each of solutions A and B. The nonoxinol oligomers elute as sharp distinct peaks, and their areas should be included in the peak response for nonoxinol 9.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and B. The sum of the areas of any peaks corresponding to nonoxynols with chain lengths n < 4 or n > 16 is not greater than 1.0% of the sum of the areas of the peaks corresponding to nonoxynols with chain lengths n = 4 to n = 16. Calculate the content of Nonoxinol 9 as a percentage, with reference to the anhydrous substance.