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Nonoxynol 9

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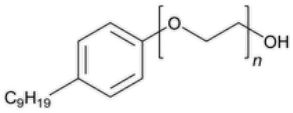

▲ (ERR 1-Jul-2022)

α-(▲4▲ (ERR 1-Jul-2022) -Nonylphenyl)-ω-hydroxynona(oxyethylene).▲▲ (ERR 1-Jul-2022)

DEFINITION

Nonoxynol 9 is an anhydrous liquid mixture consisting chiefly of mononylphenyl ethers of polyethylene glycols corresponding to the formula:

$$C_9H_{19}C_6H_4(OCH_2CH_2)_nOH$$

in which the average value of *n* is 9. It contains NLT 90.0% and NMT 110.0% of nonoxynol 9.

IDENTIFICATION

• **A. Infrared Absorption:** Its IR absorption spectrum, obtained by spreading a capillary film of it between sodium chloride plates, exhibits maxima at 1117 cm^{-1} (strong); at 1512, 1582, and 1610 cm^{-1} (medium, sharp); at 2871, 2928, and 2956 cm^{-1} (strong, unresolved); at 831 cm^{-1} (medium, broad); and at 1250 cm^{-1} (medium, sharp).

• **B.** The retention time of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **Procedure**

Mobile phase: Methanol and water (80:20)

System suitability solution: 25 mg/mL each of octoxynol 9 and USP Nonoxynol 9 RS in *Mobile phase*

Standard solution: 25 mg/mL of USP Nonoxynol 9 RS in *Mobile phase*

Sample solution: 25 mg/mL of Nonoxynol 9 in *Mobile phase*

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 3.9-mm × 25-cm; 10-µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

[Note—Nonoxynol oligomers elute as a major peak, usually with shoulders and bumps. Include these in the peak response for Nonoxynol 9.]

Analysis

Samples: *Standard solution* and *Sample solution*

Measure the responses for Nonoxynol 9, including any shoulders and bumps.

Calculate the percentage of Nonoxynol 9 in the portion of specimen taken:

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

r_U = peak response of nonoxynol 9 from the *Sample solution*

r_S = peak response of nonoxynol 9 from the *Standard solution*

C_S = concentration of USP Nonoxynol 9 RS in the *Standard solution* (mg/mL)

C_U = concentration of Nonoxynol 9 in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

• **Free Ethylene Oxide**

Stripped nonoxynol 9: Maintain Nonoxynol 9 at a temperature of 150° with constant stirring in an open vessel until it no longer displays a peak for ethylene oxide when chromatographed as directed below.

System suitability solution: 10 µg/mL each of ethylene oxide and acetaldehyde in *Stripped nonoxynol 9*

Standard stock solutions: [Note—Ethylene oxide is toxic and flammable. Prepare these solutions in a well-ventilated hood, using great care.] Chill all apparatus and reagents used in the solution of standards in a refrigerator or freezer before use. Fill a chilled pressure bottle with liquid ethylene oxide from a lecture bottle, and store in a freezer when not in use. Use a small piece of polyethylene film to protect the liquid from contact with the rubber gasket. Transfer 100 mL of chilled isopropyl alcohol to a 500-mL volumetric flask. Using a chilled graduated cylinder, transfer 25 mL of ethylene oxide to the isopropyl alcohol, and swirl gently to mix. Dilute with additional chilled isopropyl alcohol to volume, replace the stopper, and swirl gently to mix. This stock solution contains 43.6 mg/mL of ethylene oxide.

Pipet 25 mL of 0.5 N alcoholic hydrochloric acid, prepared by mixing 45 mL of hydrochloric acid with 1 L of alcohol, into a 500-mL conical flask containing 40 g of magnesium chloride hexahydrate. Shake the mixture to effect saturation. Pipet 10 mL of the ethylene oxide solution into the flask, and add 20 drops of bromocresol green TS. If the solution is not yellow (acid), add an additional volume of 0.5 N alcoholic hydrochloric acid to give an excess of 10 mL. Record the total volume of 0.5 N alcoholic hydrochloric acid added. Insert the stopper in the flask, and allow to stand for 30 min. Titrate the excess acid with 0.5 N alcoholic potassium hydroxide VS. Perform a blank titration, using 10.0 mL of isopropyl alcohol instead of ethylene oxide solution, adding the same total volume of 0.5 N alcoholic hydrochloric acid, and note the difference in volumes required. Each mL of the difference in volumes of 0.5 N alcoholic potassium hydroxide consumed is equivalent to 22.02 mg of ethylene oxide. Calculate the concentration, in mg/mL, of ethylene oxide in the stock solution. Standardize daily. Store in a refrigerator.

Prepare a 1000-ppm standard by pipeting the calculated volume (2 mL) of cold stock solution that, on the basis of the standardization, contains 88.6 mg of ethylene oxide, into a container and adding 87.0 g of *Stripped nonoxynol 9*. Prepare 10-, 5-, and 0.5-ppm standards by quantitatively diluting the 1000-ppm standard with additional *Stripped nonoxynol 9*.

Standard solutions: Transfer 5 ± 0.01 g of each *Standard stock solution* to suitable serum vials equipped with pressure-tight septum closures designed to relieve any excessive pressure, and seal them.

Sample solution: Transfer 5 ± 0.01 g of Nonoxynol 9 to a serum vial of the same kind as the vials used for the *Standard solutions*.

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 6.4-m × 2.1-mm nickel; packed with 60- to 80-mesh support S9

Temperatures

Detector: 200°

Injection port: 160°

Column: 100°

Carrier gas: Helium

Flow rate: 30 mL/min

Injection volume: 100 µL

System suitability

Samples: *System suitability solution* and *Standard solutions*

Calibration: Place the vial containing the 10-ppm ethylene oxide *Standard solution* in an oven, and heat at 90° for 30 min. Remove the vial from the oven. Using a gas-tight syringe, immediately inject a 100-µL aliquot of the headspace gas into the gas chromatograph. Obtain the area for the ethylene oxide peak (retention time about 8 min). Raise the temperature of the column to 200° after ethylene oxide elutes to volatilize heavy components. Re-equilibrate the column at 100°. Repeat the foregoing steps, using the vials containing the 5- and 0.5-ppm *Standard solution.* Plot area units versus ppm of ethylene oxide for the standards on linear graph paper, and draw the best straight line through the points.

Suitability requirements

Resolution: NLT 1.5 between ethylene oxide and acetaldehyde, *System suitability solution*

Calibration: None of the points used for constructing the straight line *Calibration* curve deviates from the line by more than 10%.

Analysis

Samples: *Standard solutions* and *Sample solution*

Place the vial containing the *Sample solution* in an oven, and heat at 90° for 30 min. Remove the vial from the oven. Immediately inject a 100-µL aliquot of the headspace gas into the gas chromatograph, and obtain the area for the ethylene oxide peak.

Calculate the concentration of ethylene oxide in the sample specimen in ppm:

$$\text{Result} = r_U \times S$$

r_U = peak area from the *Sample solution*

S = slope of the standard curve (ppm/area unit)

Acceptance criteria: NMT 1 ppm

- **Limit of Dioxane**

Apparatus: Assemble a closed-system vacuum distillation apparatus (see *Figure 1*), using glass vacuum stopcocks (*A*, *B*, and *C*). The concentrator tube (*D*)[1] is made of borosilicate or quartz (not flint) glass, graduated precisely enough to measure the 0.9 mL or more of distillate collected, and marked so that the analyst can dilute accurately to 2.0 mL.

Standard solution: 100 µg/mL of dioxane in water. Use a freshly prepared solution.

Sample solution: Transfer 20.0 g to a 50-mL round-bottom flask (*E*) having a 24/40 ground-glass neck joint. Add 1.0 mL of water. Place a small polytef-covered stirring bar in the flask, insert the stopper, and stir to mix. Immerse the flask in an ice bath, and chill for 1 min. Wrap heating tape around the tube connecting the concentrator tube (*D*) and the round-bottom flask, and apply 10 V to the tape. Apply a light coating of high-vacuum silicone grease to the ground-glass joints, and connect the concentrator tube to the 10/30 joint and the round-bottom flask to the 24/40 joint. Immerse the vacuum trap in a Dewar flask filled with liquid nitrogen, close stopcocks *A* and *B*, open stopcock *C*, and begin evacuating the system with a vacuum pump. Prepare a slurry bath from powdered dry ice and methanol, and raise the bath to the neck of the round-bottom flask. After freezing the contents of the flask for 10 min, and when the vacuum system is operating at a 0.05-mm pressure or lower, open stopcock *A* for 20 s, then close it. Remove the slurry bath, and allow the flask to warm in air for 1 min. Immerse the flask in a water bath maintained at a temperature between 20° and 25°, and after 5 min warm the water bath to between 35° and 40° (sufficient to liquefy most specimens) while stirring slowly but constantly with the magnetic bar. Cool the water in the bath by adding ice, and chill for 2 min. Replace the water bath with the slurry bath, freeze the contents of the round-bottom flask for 10 min, open stopcock *A* for 20 s, and then close it. Remove the slurry bath, and repeat the heating steps as before, this time reaching a final temperature between 45° and 50° or a temperature necessary to melt the specimen completely. If there is any condensation in the tube connecting the round-bottom flask to the concentrator tube, slowly increase the voltage to the heating tape, and heat until condensation disappears.

Stir with the magnetic stirrer throughout the following steps. Very slowly immerse the concentrator tube in a Dewar flask containing liquid nitrogen. [**Caution—**When there is liquid distillate in the concentrator tube, immerse the tube in the liquid nitrogen very slowly, or the tube will break.] Water will begin to distill into the concentrator tube. As ice forms in the concentrator tube, raise the Dewar flask to keep the liquid nitrogen level only slightly below the level of ice in the tube. When water begins to freeze in the neck of the 10/30 joint, or when liquid nitrogen reaches the 2.0-mL graduation mark on the concentrator tube, remove the Dewar flask, and allow the ice to melt without heating. After the ice has melted, check the volume of water that has distilled, and repeat the sequence of chilling and thawing until NLT 0.9 mL of water has been collected. Freeze the tube once again for 2 min, and release the vacuum first by opening stopcock *B*, followed by opening stopcock *A*. Remove the concentrator tube from the apparatus, close it with a greased stopper, and allow the ice to melt without heating. Mix the contents of the concentrator tube by swirling, note the volume of distillate, and dilute with water to 2.0 mL, if necessary.

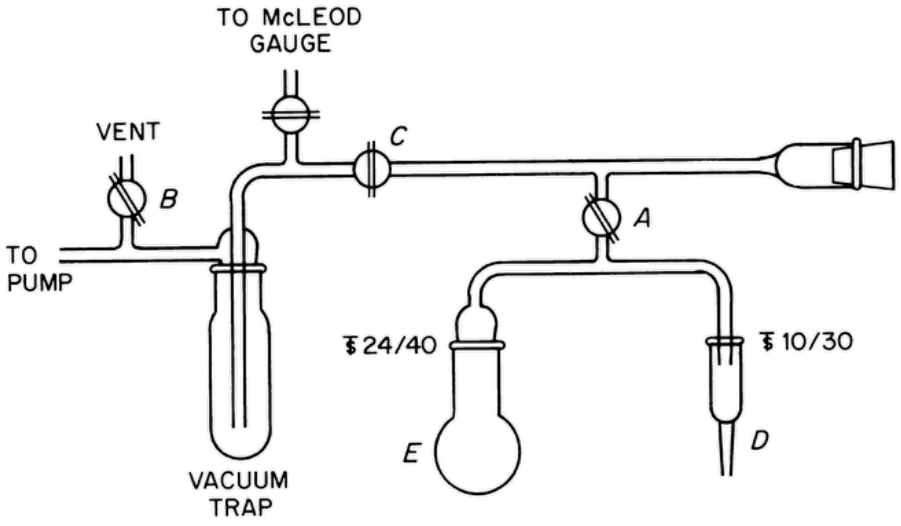

Figure 1. Closed-System Vacuum Distillation Apparatus for Dioxane

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 2-mm × 1.8-m glass; contains support S10

Temperatures

Detector: 250°

Injection port: 200°

Column: 140°

Carrier gas: Nitrogen or helium

Flow rate: 35 mL/min

Injection volume: 2–4 µL

Install an oxygen scrubber between the carrier gas line and the column. Condition the column for 72 h at 230° with 30–40 mL/min carrier flow. [NOTE—Support S10 is oxygen sensitive. Each time a column is installed, flush with carrier gas for 30–60 min before heating.]

Analysis

Samples: *Standard solution* and *Sample solution*

Acceptance criteria: The height of the peak of the *Sample solution* is NMT that of the *Standard solution*, corresponding to NMT 10 µg/g.

SPECIFIC TESTS

- **POLYETHYLENE GLYCOL**

Sample: 10 g

Analysis: Transfer the *Sample* to a 250-mL beaker. Add 100 mL of ethyl acetate, and stir on a magnetic stirrer to effect solution. Transfer, with the aid of 100 mL of 5 N sodium chloride, to a pear-shaped, 500-mL separator fitted with a glass stopper. Insert the stopper, and shake vigorously for 1 min. Remove the stopper carefully to release the pressure. Immerse a thermometer in the mixture, and support the separator so that it is partially immersed in a water bath maintained at 50°. Swirl the separator gently while letting the internal temperature rise to between 40° and 45°, then immediately remove the separator from the bath, dry the outside surface, and drain the salt (lower) layer into another pear-shaped, 500-mL separator. In the same manner, extract the ethyl acetate layer a second time with 100 mL of fresh 5 N sodium chloride, combining the two aqueous extracts. Discard the ethyl acetate layer. Wash the combined aqueous layers with 100 mL of ethyl acetate, using the same technique, and drain the salt (lower) layer into a clean pear-shaped, 500-mL separator. Discard the ethyl acetate layer. Extract the aqueous layer with two successive 100-mL portions of chloroform, draining the chloroform (lower) layers through Whatman folded filter paper 2V, and combining them into a 250-mL beaker. Evaporate on a steam bath to dryness, and continue heating until the odor of chloroform is no longer perceptible. Allow the beaker to cool. Add 25 mL of acetone, and dissolve the residue on a magnetic stirrer. Filter through Whatman folded filter paper 2V into a tared 250-mL beaker, rinsing with two 25-mL portions of acetone. Evaporate on a steam bath to dryness. Dry under vacuum at 60° for 1 h. Allow the beaker to cool, and weigh.

Acceptance criteria: NMT 1.0% of polyethylene glycol

- **CLOUD POINT**

Sample: 1.0 g

Analysis: Transfer the *Sample* to a 250-mL beaker, add 99 g of water, and mix to dissolve. Pour 30 mL of the resulting solution into a 70-mL test tube. Support the test tube in a hot water bath, and stir the contents with a thermometer constantly until the solution becomes cloudy, then remove the test tube from the bath immediately so that the temperature rises NMT 2° further, and continue stirring. The cloud point is the temperature at which the solution becomes sufficiently clear that the entire thermometer bulb is seen plainly.

Acceptance criteria: 52°–56°

- **Fats and Fixed Oils, *Acid Value*⟨401⟩:** NMT 0.2
- **Water Determination, *Method I*⟨921⟩:** NMT 0.5%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.
- **USP Reference Standards** ⟨11⟩

  USP Nonoxynol 9 RS

[1] A suitable tube is available as Chromaflex concentrator tube, Kontes Glass Co., Vineland, NJ (Catalog No. K42560-0000).

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
NONOXYNOL 9	Documentary Standards Support	CE2020 Complex Excipients
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	CE2020 Complex Excipients

Chromatographic Database Information: Chromatographic Database

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