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## Polyethylene Glycol 3350

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Poly(oxy-1,2-ethanediyl),  $\alpha$ -hydro- $\omega$ -hydroxy-;

1,2-Ethanediol, homopolymer

CAS RN<sup>®</sup>: 25322-68-3.

### DEFINITION

Polyethylene Glycol 3350 is an addition polymer of ethylene oxide and water, represented by the formula  $H(OCH_2CH_2)_nOH$ , in which  $n$

represents the average number of oxyethylene groups. The apparent weight-average molecular weight is 3015–3685 g/mol (Da). It contains NLT 97.0% and NMT 103.0% of polyethylene glycol 3350, calculated on the anhydrous basis. It may contain a suitable antioxidant.

### IDENTIFICATION

• A. **SPECTROSCOPIC IDENTIFICATION TESTS (197), Infrared Spectroscopy:** 197A or 197F. Use a thin film of test specimen, melted if necessary, in the range from 4000 to 650  $cm^{-1}$ , when the measurement is performed by using 197F.

• B. **CHROMATOGRAPHIC IDENTITY**

**Analysis:** Proceed as directed in the Assay.

**Acceptance criteria:** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*.

### ASSAY

• **PROCEDURE**

**Mobile phase:** 50  $\mu$ g/mL of sodium azide in water

**Standard solution:** 20 mg/mL of [USP Polyethylene Glycol 3350 RS](#) in *Mobile phase*

**Sample solution:** 20 mg/mL of Polyethylene Glycol 3350 in *Mobile phase*

**Chromatographic system**

(See [Chromatography \(621\), System Suitability](#).)

**Mode:** LC

**Detector:** Differential refractive index

**Columns**

**Guard:** 6-mm  $\times$  4-cm; 6- $\mu$ m packing L25

**Analytical:** 7.8-mm  $\times$  30-cm; 6- $\mu$ m packing L25

**Temperatures**

**Detector:** 35  $\pm$  1°

**Column:** 35  $\pm$  1°

**Flow rate:** 0.8 mL/min

**Injection volume:** 20  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

[NOTE—The retention time for polyethylene glycol 3350 is about 8.5 min.]

**Suitability requirements**

**Relative standard deviation:** NMT 1.5%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of polyethylene glycol 3350 [ $H(OCH_2CH_2)_nOH$ ] in the portion of Polyethylene Glycol 3350 taken:

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

$r_u$  = peak response from the *Sample solution* $r_s$  = peak response from the *Standard solution* $C_s$  = concentration of [USP Polyethylene Glycol 3350 RS](#) in the *Standard solution* (mg/mL) $C_u$  = concentration of Polyethylene Glycol 3350 in the *Sample solution* (mg/mL)**Acceptance criteria:** 97.0%–103.0% on the anhydrous basis**IMPURITIES**• [RESIDUE ON IGNITION \(281\)](#)**Sample:** 2 g**Analysis:** Proceed as directed, moistening the residue with 2 mL of sulfuric acid.**Acceptance criteria:** NMT 0.1%• [LIMIT OF ETHYLENE OXIDE AND DIOXANE](#)**Analysis:** Proceed as directed in [Ethylene Oxide and Dioxane \(228\), Method II](#).**Acceptance criteria****Ethylene oxide:** NMT 1 µg/g**Dioxane:** NMT 10 µg/g• [LIMIT OF ETHYLENE GLYCOL AND DIETHYLENE GLYCOL](#)**Mobile phase:** 50 µg/mL of sodium azide in water**Eluant:** Water**Standard stock solution:** 10 mg/g of [USP Diethylene Glycol RS](#) and 10 mg/g of [USP Ethylene Glycol RS](#) in *Eluant***Standard solution:** Transfer 0.1 g of *Standard stock solution* to a 100-mL volumetric flask. Dilute with water to volume. The *Standard solution* contains 0.01 mg/mL of [USP Diethylene Glycol RS](#) and 0.01 mg/mL of [USP Ethylene Glycol RS](#).**Sample solution:** 10 mg/mL of Polyethylene Glycol 3350 in *Eluant***Chromatographic system**(See [Chromatography \(621\), System Suitability](#).)**Mode:** LC**Detector:** Differential refractive index**Column:** 7.8-mm × 30-cm; 7-µm packing L89, 125-Å pore size**Temperatures****Detector:** 35 ± 1°**Column:** 35 ± 1°**Flow rate:** 0.5 mL/min**Injection volume:** 100 µL**Run time:** 30 min**System suitability****Sample:** *Standard solution*

[NOTE—The relative retention times for diethylene glycol and ethylene glycol are 1.0 and 1.1, respectively.]

**Suitability requirements****Resolution:** NLT 0.9 between diethylene glycol and ethylene glycol**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of diethylene glycol (or ethylene glycol) in the portion of Polyethylene Glycol 3350 taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

 $r_u$  = peak response of diethylene glycol (or ethylene glycol) from the *Sample solution* $r_s$  = peak response of diethylene glycol (or ethylene glycol) from the *Standard solution* $C_s$  = concentration of [USP Diethylene Glycol RS](#) (or [USP Ethylene Glycol RS](#)) in the *Standard solution* (mg/mL) $C_u$  = concentration of Polyethylene Glycol 3350 in the *Sample solution* (mg/mL)**Acceptance criteria****Ethylene glycol:** NMT 0.062%

Sum of diethylene glycol and ethylene glycol: NMT 0.2%

**Change to read:**

- **LIMIT OF FORMALDEHYDE AND ACETALDEHYDE**

**Solution A:** Water**Solution B:** Acetonitrile**Mobile phase:** See [Table 1](#).**Table 1**

Time <sup>a</sup> (min)	Solution A (%)	Solution B (%)
0	50	50
11	0	100

<sup>a</sup> The equilibration time is 5 min.

[NOTE—Use amber containers and amber autosampler vials.]

**2,4-DNPH solution:** Transfer 250 mg of 2,4-dinitrophenylhydrazine (2,4-DNPH) to a 50-mL volumetric flask, add 20.0 mL of acetonitrile, and swirl to mix. Add 3.0 mL of hydrochloric acid to the flask, and swirl to mix. Sonicate until all solids are dissolved, and dilute with acetonitrile to volume.

**Formaldehyde-2,4-DNPH solution:** 100 µg/mL of aldehyde equivalent in acetonitrile<sup>1</sup>

**Acetaldehyde-2,4-DNPH solution:** 1000 µg/mL of aldehyde equivalent in acetonitrile<sup>2</sup>

**Formaldehyde stock solution:** Transfer 500 µL of *Formaldehyde-2,4-DNPH solution* to a 10-mL volumetric flask. Dilute with acetonitrile to volume. The formaldehyde concentration is 5.0 µg/mL.

**Acetaldehyde stock solution:** Transfer 500 µL of *Acetaldehyde-2,4-DNPH solution* to a 10-mL volumetric flask. Dilute with acetonitrile to volume. The acetaldehyde concentration is 50.0 µg/mL.

**Standard solution:** Transfer 1.5 mL of *Formaldehyde stock solution* and 1.2 mL of *Acetaldehyde stock solution* to a 10-mL volumetric flask.

Dilute with acetonitrile to volume, and mix well. The concentrations of formaldehyde and acetaldehyde are 0.75 and 6.0 µg/mL, respectively.

**Sample solution:** Transfer 0.5 g of Polyethylene Glycol 3350 to a 10-mL volumetric flask. Add 1.0 mL of acetonitrile to the flask, and swirl to dissolve the sample. Add 2.0 mL of *2,4-DNPH solution* to the flask, and swirl to mix. Allow the solution to react for 15 min, then dilute with acetonitrile to volume.

**Chromatographic system**

(See [Chromatography \(621\), System Suitability](#).)

**Mode:** LC

**Detector:** UV 360 nm

**Column:** 3.0-mm × 15-cm; 3.5-µm packing L7, 80-Å pore size

**Column temperature:** 30 ± 1°

**Flow rate:** 0.65 mL/min

**Injection volume:** 5 µL

**System suitability**

**Sample:** *Standard solution*

[NOTE—The relative retention times for formaldehyde and acetaldehyde are 1.0 and 1.2, respectively.]

**Suitability requirements**

**Resolution:** NLT 2.0 between formaldehyde and acetaldehyde

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the content of formaldehyde (or acetaldehyde), in µg/g, in the portion of Polyethylene Glycol 3350 taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u)$$

$r_u$  = peak response of formaldehyde (or acetaldehyde) from the *Sample solution*

$r_s$  = peak response of formaldehyde (or acetaldehyde) from the *Standard solution*

$C_s$  = concentration of formaldehyde (or acetaldehyde) in the *Standard solution* (µg/mL)

**Acceptance criteria****Formaldehyde:** NMT  $\Delta$  30  $\mu\text{g/g}$   $\Delta$  (RB 1-Dec-2020)**Sum of formaldehyde and acetaldehyde:** NMT 200  $\mu\text{g/g}$ **SPECIFIC TESTS****• APPARENT WEIGHT-AVERAGE MOLECULAR WEIGHT AND POLYDISPERSITY****Mobile phase:** Water

**Standard solutions:** Prepare 1.0 mg/mL each of five [polyethylene glycol standards with molecular weights of 1000, 2000, 3000, 4000, and 6000 Daltons \(g/mol\)](#) in *Mobile phase* separately in five individual flasks. Pass a portion of each solution through a 0.45- $\mu\text{m}$  polytetrafluoroethylene (PTFE) syringe filter.<sup>3</sup> Discard the first 2 mL, and transfer the solution for analysis.

**Sample solution:** 1.0 mg/mL of Polyethylene Glycol 3350 in *Mobile phase*. Pass a portion of the solution through a 0.45- $\mu\text{m}$  PTFE syringe filter.<sup>3</sup> Discard the first 2 mL, and transfer the solution for analysis.

**Chromatographic system**(See [Chromatography \(621\), System Suitability](#).)**Mode:** LC**Detector:** Differential refractive index**Column:** 7.8-mm  $\times$  30-cm; 6- $\mu\text{m}$  packing L37**Temperatures****Detector:**  $35 \pm 1^\circ$ **Column:**  $35 \pm 1^\circ$ **Flow rate:** 0.8 mL/min**Injection volume:** 10  $\mu\text{L}$ **Run time:** 18 min**Analysis****Samples:** *Standard solutions* and *Sample solution*

Separately inject equal volumes of the *Standard solutions* and the *Sample solution* into the chromatograph, record the chromatograms, and determine the elution peak maxima and the corresponding retention times for the five polyethylene glycol standards.

**Calibration curve:** Plot the retention times on the x-axis against the  $\log M_p$  ( $M_p$ : peak molecular weights) on the y-axis for the peaks from the polyethylene glycol standard to generate a calibration curve using suitable gel permeation chromatography or size exclusion chromatography (GPC/SEC) software.

**Calculations:** Compute the data using the same GPC/SEC software, and determine the number- and weight-average molecular weights,  $M_N$  and  $M_W$ , in g/mol (Da), respectively, for the chromatogram of the *Sample solution*.

Calculate the polydispersity for Polyethylene Glycol 3350:

$$\text{Result} = M_W/M_N$$

$M_W$  = weight-average molecular weight from the *Sample solution* (g/mol)

$M_N$  = number-average molecular weight from the *Sample solution* (g/mol)

**Acceptance criteria:** The value of apparent weight-average molecular weight is 3015–3685 g/mol. Polydispersity is 90%–110% of the value stated on the label or within the range indicated on the label.

**• HYDROXYL VALUE**

**Phthalic anhydride solution:** Place 49.0 g of phthalic anhydride into an amber bottle, and dissolve in 300 mL of pyridine from a freshly opened bottle or pyridine that has been freshly distilled over phthalic anhydride. Shake vigorously until completely dissolved. Add 7 g of imidazole, swirl carefully to dissolve, and allow to stand for 16 h before using.

**Sample solution:** Carefully introduce 25.0 mL of *Phthalic anhydride solution* into a dry, heat-resistant pressure bottle. Add 12.0 g of Polyethylene Glycol 3350. Add 25 mL of pyridine, from a freshly opened bottle or pyridine that has been freshly distilled over phthalic anhydride. Swirl to dissolve, insert the stopper in the bottle, and wrap it securely in a cloth bag.

**Blank:** 25.0 mL of *Phthalic anhydride solution* plus any additional pyridine added to the bottle

**Analysis:** Immerse the bottle in a water bath maintained at a temperature between 96° and 100°, to the same depth as that of the mixture in the bottle. Remove the bottles from the bath after 5 min and, without unwrapping, swirl for 30 s to homogenize. Heat in the water bath for 60 min, then remove from the bath, and allow it to cool to room temperature. Uncap the bottle carefully to release any pressure, remove

from the bag, add 10 mL of water, and swirl thoroughly. Wait 2 min, add 0.5 mL of a solution of phenolphthalein in pyridine (1 in 100), and titrate with 0.5 N sodium hydroxide VS to the first pink color that persists for 15 s. Perform a blank determination.

Calculate the hydroxyl value:

$$\text{Result} = [M_r \times (V_B - V_S) \times N] / W$$

$M_r$  = molecular weight of potassium hydroxide, 56.11

$V_B$  = volume of 0.5 N sodium hydroxide consumed in the blank test (mL)

$V_S$  = volume of 0.5 N sodium hydroxide consumed in the actual test (mL)

$N$  = exact normality of the sodium hydroxide solution

$W$  = weight of Polyethylene Glycol 3350 taken for the test (g)

**Acceptance criteria:** 30–38

• **ACIDITY AND ALKALINITY**

**Sample solution:** Dissolve 5.0 g of Polyethylene Glycol 3350 in 100 mL of carbon dioxide-free water.

**Analysis:** Add 0.3 mL of a saturated solution of potassium chloride into the *Sample solution*. The test solution should be maintained at  $25 \pm 2^\circ$  during the measurement. Measure the pH following [pH \(791\)](#).

**Acceptance criteria:** 4.5–7.5

• [WATER DETERMINATION \(921\), Method I](#)

**Sample:** 2.0 g

**Acceptance criteria:** NMT 1.0%

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in tight containers, protected from direct sunlight. Avoid exposure to excessive heat.

• **LABELING:** Label it to indicate its polydispersity ( $M_w/M_n$ ) or its polydispersity range. Label it to indicate the name and amount of any added antioxidant.

• [USP REFERENCE STANDARDS \(11\)](#)

[USP Diethylene Glycol RS](#)

[USP Ethylene Glycol RS](#)

[USP Polyethylene Glycol 3350 RS](#)

<sup>1</sup> Available from Sigma-Aldrich Corporation, or equivalent.

<sup>2</sup> Available from Sigma-Aldrich Corporation, or equivalent.

<sup>3</sup> Millipore® Millex® LCR HPLC syringe filters with hydrophilic PTFE membrane is suitable, or any other equivalent filter.

**Auxiliary Information** - Please [check for your question in the FAQs](#) before contacting USP.

Topic/Question	Contact	Expert Committee
POLYETHYLENE GLYCOL 3350	<a href="#">Documentary Standards Support</a>	CE2020 Complex Excipients
REFERENCE STANDARD SUPPORT	RS Technical Services <a href="mailto:RSTECH@usp.org">RSTECH@usp.org</a>	CE2020 Complex Excipients

**Chromatographic Database Information:** [Chromatographic Database](#)

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