Polysorbate 80

Portions of the monograph text that are national USP text, and are not part of the harmonized text, are marked with symbols (•) to specify this fact.

Sorbital, mono-9-octadecenoate, poly(oxy-1,2-ethanediyl) derivs., (Z)-; Polyoxyethylene 20 sorbitan monooleate [9005-65-6].

**DEFINITION**

Polysorbate 80 is a mixture of partial esters of fatty acids, mainly oleic acid, with sorbitol and its anhydrides ethoxylated with approximately 20 moles of ethylene oxide for each mole of sorbitol and sorbitol anhydrides.

**IDENTIFICATION**

- A. It meets the requirements of the test for Composition of Fatty Acids.

**ASSAY**

- **COMPOSITION OF FATTY ACIDS**

  Diluent: 20 g/L of sodium hydroxide in methanol

  Boron trifluoride–methanol solution: 140 g/L of boron trifluoride in methanol

  Saturated sodium chloride solution: Sodium chloride and water (1:2). Before use, decant the solution from any undissolved substance and filter, if necessary.

  Reference solution A: Prepare 0.50 g of the mixture of calibrating substances with the composition described in Table 1. Dissolve in heptane, and dilute with heptane to 50.0 mL.

  Reference solution B: Reference solution A in heptane (1 in 10)

  Reference solution C: Prepare 0.50 g of a mixture of fatty acid methyl esters, which corresponds to the composition of the substance to be examined. Dissolve in heptane, and dilute with heptane to 50.0 mL.

  Sample solution: Dissolve 0.10 g of Polysorbate 80 in 2 mL of Diluent in a 25-mL conical flask, and boil under a reflux condenser for 30 min. Add 2.0 mL of Boron trifluoride–methanol solution through the condenser, and boil for 5 min. Cool, add 10.0 mL of Saturated sodium chloride solution, shake for about 15 s, and add a quantity of Saturated sodium chloride solution such that the upper phase is brought into the neck of the flask. Collect 2 mL of the upper phase, wash with three quantities, each of 2 mL, of water, and dry over anhydrous sodium sulfate.

**Table 1 (Continued)**

<table>
<thead>
<tr>
<th>Mixture of the Following Substances</th>
<th>Composition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl myristate</td>
<td>5</td>
</tr>
<tr>
<td>Methyl palmitate</td>
<td>10</td>
</tr>
<tr>
<td>Methyl stearate</td>
<td>15</td>
</tr>
<tr>
<td>Methyl arachidate</td>
<td>20</td>
</tr>
<tr>
<td>Methyl oleate</td>
<td>20</td>
</tr>
<tr>
<td>Methyl eicosenate</td>
<td>10</td>
</tr>
<tr>
<td>Methyl behenate</td>
<td>10</td>
</tr>
<tr>
<td>Methyl lignocerate</td>
<td>10</td>
</tr>
</tbody>
</table>

**Chromatographic system**

(See Chromatography (621), System Suitability.)

- **Mode**: GC
- **Detector**: Flame ionization
- **Column**: See Table 2.

**Table 2**

<table>
<thead>
<tr>
<th>Initial Temperature ()</th>
<th>Temperature Ramp ()/min</th>
<th>Final Temperature ()</th>
<th>Hold Time at Final Temperature (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>10</td>
<td>220</td>
<td>40</td>
</tr>
<tr>
<td>220</td>
<td>—</td>
<td>220</td>
<td>—</td>
</tr>
</tbody>
</table>

Carrier gas: Helium

Linear velocity: 50 cm/s

Injection volume: 1 μL

**Suitability requirements**

- **Resolution**: NLT 1.8 between the peaks due to methyl oleate and methyl stearate, Reference solution A

- **Theoretical plates**: NLT 30,000 calculated for the peak of methyl stearate, Reference solution A

- **Signal-to-noise ratio**: NLT 5 for the peak of methyl myristate, Reference solution B

**Analysis**

- **Sample**: Sample solution

Identify the peaks from Reference solution C. Calculate the percentage of each component in the Sample solution:

\[ \text{Result} = \left( \frac{A_i}{A_r} \right) \times 100 \]

**Acceptance criteria**: See Table 3.
**ETHYLENE OXIDE AND DIOXANE**

**Residue on Ignition**

**Sample**: 2.00 g

**Analysis**: Heat a silica or platinum crucible to redness for 30 min, allow to cool in a desiccator, and weigh. Equally distribute the sample in the crucible. Dry at 100°–105° for 1 h and ignite to constant mass in a muffle furnace at 600 ± 25°, allowing the crucible to cool in a desiccator after each ignition. Flames should not be produced at any time during the procedure. If after prolonged ignition the ash still contains black particles, take up with hot water, pass through an ashless filter paper, and ignite the residue and the filter paper. Combine the filtrate with the ash, carefully evaporate to dryness, and ignite to constant mass.

**Acceptance criteria**: NMT 0.25%.

**Delete the following:**

- **Heavy Metals, Method II (231)**: NMT 10 ppm.

**Impurities**

**Table 4 (Continued)**

<table>
<thead>
<tr>
<th>Initial Temperature (°C)</th>
<th>Temperature Ramp (°C/min)</th>
<th>Final Temperature (°C)</th>
<th>Hold Time at Final Temperature (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>250</td>
<td>250</td>
<td>250</td>
<td>5</td>
</tr>
</tbody>
</table>

**Split ratio**: 3:5:1

**Carrier gas**: Helium

**Flow rate**: 4.0 mL/min

**Injection volume**: 1 mL

**System suitability**

**Sample**: Reference solution

**Resolution**: NLT 2.0 between the peaks due to acetaldehyde and ethylene oxide.

**Analysis**

**Samples**: Sample solution A and Sample solution B

Calculate the content of ethylene oxide:

\[
\text{Result} = \left( \frac{2 \times C_{\text{EO}} \times A_{l}}{A_{l} - A_{s}} \right) \]

**Acetalddehyde standard solution**: 0.01 mg/mL of acetalddehyde in water

**Standard solution**: Dilute 6.0 mL of **Ethylene oxide standard solution** and 2.5 mL of **Dioxane standard solution** with water to 25.0 mL.

**Sample solution A**: Transfer 1.0 g of Polysorbate 80 to a 10-mL headspace vial. Add 2.0 mL of water, and seal the vial immediately with a Teflon-coated, silicon membrane and an aluminum cap.

**Sample solution B**: Transfer 1.0 g of Polysorbate 80 to a 10-mL headspace vial. Add 2.0 mL of the standard solution, and seal the vial immediately with a Teflon-coated, silicon membrane and an aluminum cap.

**Reference solution**: Transfer 2.0 mL of Acetalddehyde standard solution and 2.0 mL of Ethylene oxide standard solution to a 10-mL headspace vial, and seal the vial immediately with a Teflon-coated, silicon membrane and an aluminum cap.

**Chromatographic system**

(See Chromatography (621), System Suitability.)

**Mode**: Headspace GC

**Detector**: Flame ionization

**Column**: 0.53-mm × 50-m G27 on fused silica; film thickness 5 µm

**Temperatures**

**Injection port**: 85°

**Detector**: 250°

**Column**: See Table 4.

** SPECIFIC TESTS**

- **Specific Gravity (841)**: 1.06–1.09.

**Change to read:**

- **Viscosity—Capillary Methods (911) or Viscosity—Rotational Methods (912)**: 300–500 centistokes at 25°.

**Change to read:**

- **Fats and Fixed Oils, Acid Value (401)**

  **Light petroleum**: It has the following properties: a clear, colorless, flammable liquid without fluorescence; practically insoluble in water; miscible with alcohol; density at 20° about 0.720; distillation range 100°–120°; water content NMT 0.03%.

  **Sample solution**: Dissolve 5.0 g in 50 mL of a mixture of equal volumes of alcohol and Light petroleum (previously neutralized with 0.1 N potassium hydroxide or 0.1 N sodium hydroxide), using 0.5 mL of phenolphthalein TS as the indicator. If necessary, heat to about 90° to dissolve the substance to be examined.

  **Petroleum ether**: boiling range 100°–140°; [CAS 64742-49-0] from Fisher Scientific; catalog number AC23302-0025 is suitable.
Analysis: Titrate the Sample solution with 0.1 N potassium hydroxide VS or 0.1 N sodium hydroxide VS until the pink color persists for at least 15 s. When heating has been applied to aid dissolution, maintain the temperature at about 90° during the titration.

Acceptance criteria: NMT 2.0

**FATS AND FIXED OILS, Hydroxyl Value (401)**

Sample: 2.0 g

Analysis: Transfer the Sample into a 100-mL beaker, and dissolve with 20 mL of glacial acetic acid. Add 1 mL of Saturated potassium iodide solution, and allow to stand for 1 min. Add 50 mL of carbon dioxide-free water and a magnetic stirring bar. Titrate with 0.01 M sodium thiosulfate VS, determining the endpoint potentiometrically. Carry out a blank titration.

Acceptance criteria: NMT 10

**FATS AND FIXED OILS, Saponification Value (401)**

Sample: 4.0 g

Analysis: Transfer the Sample into a 150-mL acetylation flask fitted with an air condenser. Add 5.0 mL of Pyridine–Acetic Anhydride Reagent, and attach the air condenser. Heat the flask in a water bath for 1 h keeping the level of the water about 2.5 cm above the level of the liquid in the flask. Withdraw the flask, and allow to cool. Add 5 mL of water through the upper end of the condenser. If a cloudiness appears, add sufficient pyridine to clear it, noting the volume added. Shake the flask, and replace in the water bath for 10 min. Withdraw the flask, and allow to cool. Rinse the condenser and the walls of the flask with 5 mL of alcohol, previously neutralized with phenolphthalein TS. Titrate with 0.5 N alcoholic potassium hydroxide VS using 0.2 mL of phenolphthalein TS as the indicator. Carry out a blank test under the same conditions.

Acceptance criteria: 65–80

**FATS AND FIXED OILS, Peroxide Value (401)**

Sample: 10.0 g

Saturated potassium iodide solution: Prepare a saturated solution of potassium iodide in carbon dioxide-free water. Make sure the solution remains saturated as indicated by the presence of undissolved crystals.

Analysis: Transfer the Sample into a 250-mL borosilicate glass flask fitted with a reflux condenser. Add 30.0 mL of 0.5 N alcoholic potassium hydroxide VS and a few glass beads. Attach the condenser, and heat under reflux for 60 min. Add 1 mL of phenolphthalein TS and 50 mL of dehydrated alcohol, and titrate immediately with 0.5 N hydrochloric acid VS. Carry out a blank test under the same conditions.

Acceptance criteria: 45–55

**WATER DETERMINATION, Method I (921):** NMT 3.0%, determined on 1.0 g

**ADDITIONAL REQUIREMENTS**

**Packaging and Storage:** Store in an airtight container, protected from light.

**USP Reference Standards (11)**

USP Polysorbate 80 RS.