BRIEFING

Polysorbate 80. The European Pharmacopoeia is the coordinating pharmacopeia for the international harmonization of the compendial standards for the Polysorbate 80 monograph, as part of the process of international harmonization of monographs and general analytical methods of the European, Japanese, and United States pharmacopeias. The following monograph, which represents the **ADOPTION STAGE 6** document, is based on the corresponding monograph for Polysorbate 80 that was prepared by the European Pharmacopoeia and published in *PF* 33(5) [Sept.–Oct. 2007]. The European Pharmacopoeia draft was based in part on comments from the Japanese Pharmacopoeia and the United States Pharmacopeia in response to the Provisional Harmonized Text Stage 5A and 5B drafts prepared by the European Pharmacopoeia. No significant differences were made from the draft published in *PF* 33(5) [Sept.–Oct. 2007].

(EM2: K. Moore.) RTS—C65925

Add the following:

▲Polysorbate 80

Attributes	EP	JP	USP
Definition	+	+	+
Characters (Description and Solubility, Specific Gravity, Viscosity)	+	+	+
Identification (Composition of Fatty Acids)	+	+	+
Acid Value	+	+	+
Hydroxyl Value	+	+	+
Peroxide Value	+	+	+
Saponification Value	+	+	+
Composition of Fatty Acids	+	+	+
Ethylene Oxide and Dioxane	+	+	+
Water	+	+	+
Residue on Ignition	+	+	+
Storage	+	+	+

Legend: + will adopt and implement; – will not stipulate
Nonharmonized attributes: *Identification* by IR (EP), *Heavy Metals* (USP)
Each pharmacopeia will adapt the text to take account of local reference substances and spectra and reagent specifications.

Sorbitan, 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

• **PROCEDURE**
Acceptance criteria: It complies with the test for *Composition of Fatty Acids*.

ASSAY

• **COMPOSITION OF FATTY ACIDS**
Diluent: 20 g/L sodium hydroxide 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. [NOTE—Commercially available mixtures of fatty acid methyl esters may also be used.]
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 14% boron trifluoride–methanol through the condenser, and boil for 30 min. Add 4 mL of heptane through the condenser, and boil for 5 min. Cool and 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

Mixture of the Following Substances	Composition (%)
Methyl myristate	5
Methyl palmitate	10
Methyl stearate	15
Methyl arachidate	20
Methyl oleate	20
Methyl eicosenoate	10
Methyl behenate	10
Methyl lignocerate	10

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.32-mm × 30-m G16 on fused silica, film thickness 0.5 µm
Column temperature: See the temperature program table below.

	Time (min)	Temperature (°)
Detector temperature		250
Injection port		250
Column	0	80
	14	220
	54	220

Carrier gas: Helium
Linear velocity: 50 cm/s
Injection size: 1 µL
System suitability
Samples: *Reference solution A* and *Reference solution B*
Suitability requirements
Resolution: NLT 1.8 between the peaks due to methyl oleate and methyl stearate, *Reference solution A*
Signal-to-noise ratio: NLT 5 for the peak of methyl myristate, *Reference solution B*
Theoretical plates: NLT 30,000 calculated for the peak of methyl stearate, *Reference solution A*

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

$$\text{Result} = A_c/A_T \times 100$$

A_c = peak area for the component of interest
A_T = total area of all peaks related to fatty acids

Acceptance criteria: Myristic acid, NMT 5.0%; Palmitic acid, NMT 16.0%; Palmitoleic acid, NMT 8.0%; Stearic acid, NMT 6.0%; Oleic acid, NLT 58.0%; Linoleic acid, NMT 18.0%; Linolenic acid, NMT 4.0%

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION ⟨281⟩:** Heat a silica or platinum crucible to redness for 30 min, allow to cool in a desiccator, and weigh. Evenly distribute 2.00 g of the substance to be examined in the crucible. Dry at 100° to 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, filter 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%
- **‡HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 10 ppm‡

Organic Impurities

- **PROCEDURE: ETHYLENE OXIDE AND DIOXANE**
  Ethylene oxide standard solution: Dilute 0.5 mL of a commercially available solution of ethylene oxide in methylene chloride (50 mg/mL) with water to 50.0 mL. [NOTE—The solution is stable for 3 months, if stored in vials with a teflon-coated, silicon membrane crimped caps at –20°.] Allow to reach room temperature. Dilute 1.0 mL of this solution with water to 250.0 mL.
  Dioxane standard solution: Dioxane in water (v/v) 1 in 20,000
  Acetaldehyde standard solution: 0.01 mg/mL acetaldehyde 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: 1.0 g of Polysorbate 80 into 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: 1.0 g of Polysorbate 80 into a 10-mL headspace vial. Add 2.0 mL of *Standard solution*, and seal the vial immediately with a teflon-coated, silicon membrane and an aluminum cap.
  Reference solution: Introduce 2.0 mL of *Acetaldehyde 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[1]**:** 0.53-mm × 50-m G27 on fused silica, film thickness 5 µm
  Column temperature: See the temperature program table below.

	Time (min)	Temperature (°)
Detector temperature		250
Injection port		85
Column	0	70
	18	250
	23	250

  Split ratio: 3.5
  Carrier gas: Helium
  Flow rate: 4.0 mL/min
  Injection size: 1 mL
  [NOTE—The relative retention times for ethylene oxide, acetaldehyde, and dioxane are 1.0, 0.9, and 1.9, respectively. The retention time for ethylene oxide is about 6.5 min.]
  System suitability
  Sample: *Reference solution*
  Suitability requirements
  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:

$$\text{Result} = (2 \times C_{EO} \times A_a)/(A_b - A_a)$$

C_{EO} = concentration of ethylene oxide in *Sample solution A* (µg/mL)
A_a = peak area of ethylene oxide from *Sample solution A*
A_b = peak area of ethylene oxide from *Sample solution B*

  Calculate the content of dioxane:

$$\text{Result} = (2 \times 1.03 \times C_D \times A_{a'})/(A_{b'} - A_{a'})$$

1.03 = density of dioxane (g/mL)
C_D = concentration of dioxane in *Sample solution A* (µg/mL)
$A_{a'}$ = peak area of dioxane from *Sample solution A*
$A_{b'}$ = peak area of dioxane from *Sample solution B*

  Acceptance criteria: NMT 1 ppm for ethylene oxide; NMT 10 ppm for dioxane

SPECIFIC TESTS

- **SPECIFIC GRAVITY ⟨841⟩:** about 1.10 at 20°
- **VISCOSITY ⟨911⟩:** about 400 mPa · s at 25°
- **FATS AND FIXED OILS,** *Acid Value* ⟨**401**⟩
  Sample solution: Dissolve 5.0 g in 50 mL of a mixture of equal volumes of alcohol and hexane (previously neutralized with 0.1 N potassium hydroxide or 0.1 N sodium hydroxide), using 0.5 mL of phenolphthalein solution as indicator. If necessary, heat to about 90° to dissolve the substance to be examined.
  Analysis: Titrate the *Sample solution* with 0.1 N potassium hydroxide or 0.1 N sodium hydroxide 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: Introduce 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

[1] CP-Sil 8 CB is suitable.

phenolphthalein solution. Titrate with 0.5 N alcoholic potassium hydroxide using 0.2 mL of phenolphthalein solution as 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: Introduce 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, 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: Introduce 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 and a few glass beads. Attach the condenser, and heat under reflux for 60 min. Add 1 mL of phenolphthalein solution and 50 mL of dehydrated alcohol, and titrate immediately with 0.5 N hydrochloric acid. 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.▲*NF28*

Stage 6 Harmonization