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.

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

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

Change to read:

- **▲◆▲NF34B. INFRARED ABSORPTION ⟨197F⟩▲◆▲NF34**

ASSAY

Change to read:

- **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▲NF34

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 *▲Boron trifluoride–methanol solution▲NF34* through the condenser, and boil for 30 min. Add 4 mL of heptane 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

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
Temperatures
Injection port: 250°
Detector: 250°
Column: See *Table 2*.

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
80	10	220	—
220	—	220	40

Carrier gas: Helium
Linear velocity: 50 cm/s
Injection volume: 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*
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*:

$$\text{Result} = (A_C/A_T) \times 100$$

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

Acceptance criteria: See *Table 3*.

Table 3

Name	Acceptance Criteria, NMT (%)	Acceptance Criteria, NLT (%)
Myristic acid	5.0	—
Palmitic acid	16.0	—
Palmitoleic acid	8.0	—
Stearic acid	6.0	—
Oleic acid	—	58.0
Linoleic acid	18.0	—
Linolenic acid	4.0	—

IMPURITIES

- **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. Evenly 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◆•• (Official 1-Dec-2015)

- **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 and 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 of 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: 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 *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
  Temperatures
  Injection port: 85°
  Detector: 250°
  Column: See *Table 4*.

Table 4

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
70	10	250	—

[1] CP-Sil 8 CB is suitable.

Table 4 *(Continued)*

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
250	—	250	5

Split ratio: 3.5: 1
Carrier gas: Helium
Flow rate: 4.0 mL/min
Injection volume: 1 mL
System suitability
Sample: *Reference solution*
[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.]
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 B* (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:

$$\text{Result} = (2 \times D \times C_D \times A_{A'})/(A_{B'} - A_{A'})$$

D = density of dioxane, 1.03 g/mL
C_D = concentration of dioxane in *Sample solution B* (µL/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**⟩**:** 1.06–1.09◆

Change to read:

- ◆•**VISCOSITY—CAPILLARY METHODS** ⟨**911**⟩ or **VISCOSITY—ROTATIONAL METHODS** ⟨**912**⟩**:**• (CN 1-May-2015) 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%.[2] ▲*NF34*
  Sample solution: Dissolve 5.0 g in 50 mL of a mixture of equal volumes of alcohol and ▲*Light petroleum*▲*NF34* (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.

▲[2] Petroleum ether; boiling range 100°–140°; [CAS 64742-49-0] from Fisher Scientific; catalog number AC23302-0025 is suitable.▲*NF34*

Analysis: Titrate the *Sample solution* •with 0.1 N potassium hydroxide VS or 0.1 N sodium hydroxide VS• (ERR 1-Dec-2014) 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 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 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 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◆