Total ash ⟨561⟩**:** not more than 0.1%.

Limit of free glycerin—

Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay*.

Standard solutions—Prepare four solutions by dissolving accurately weighed quantities of glycerin in tetrahydrofuran and by diluting each with tetrahydrofuran, as necessary, to obtain solutions having known concentrations of about 0.4, 1.0, 2.0, and 4.0 mg per mL.

Test solution—Use the *Assay preparation,* prepared as directed in the *Assay*.

Procedure—Separately inject equal volumes (about 40 µL) of the *Standard solutions* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the glycerin peaks. Plot the glycerin peak responses obtained versus the concentration, in mg per mL, of glycerin in the *Standard solutions*. From the standard curve so obtained, determine the glycerin concentration, *C*, in mg per mL, in the *Test solution*. Calculate the percentage of free glycerin in the portion of Glyceryl Monolinoleate taken by the formula:

$$500(C/W)$$

in which *C* is as obtained above; and *W* is the amount, in mg, of Glyceryl Monolinoleate taken to prepare the *Test solution:* not more than 6.0% of free glycerin is found.

Assay—

Mobile phase: tetrahydrofuran.

Assay preparation—Transfer about 200 mg of Glyceryl Monolinoleate, accurately weighed, to a 5-mL volumetric flask, dissolve in and dilute with tetrahydrofuran to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—Prepare as directed in the *Assay* under *Glyceryl Distearate*. Chromatograph the *Assay preparation*, and record the peak responses as directed for *Procedure:* the relative retention times are about 1.0 for glycerin, 0.86 for monoglycerides, 0.80 for diglycerides, and 0.76 for triglycerides; the resolution, *R,* between the diglycerides and monoglycerides is not less than 1.0; and the relative standard deviation for replicate injections determined from the monoglycerides peak is not more than 2.0%.

Procedure—Inject a volume (about 40 µL) of the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of monoglycerides, diglycerides, and triglycerides in the portion of Glyceryl Monolinoleate taken by the formula:

$$100(r_i / r_s)$$

in which r_i is the individual peak response for the monoglycerides, diglycerides, and triglycerides, as appropriate; and r_s is the sum of the responses for all of the glyceride peaks.

Glyceryl Monooleate

Oleic acid, 2,3-dihydroxypropyl ester, (±); (*RS*)-1-Glyceryl oleate 356.54
[25496-72-4].

DEFINITION

Glyceryl Monooleate is a mixture of monoglycerides, mainly glyceryl monooleate, together with variable quantities of diglycerides and triglycerides. It is obtained by partial glycerolysis of vegetable oil that consists mainly of triglycerides of oleic acid, or by esterification of glycerol with oleic acid of vegetable or animal origin. It is defined by the nominal content of monoglycerides. The assay requirements differ as set forth in the accompanying table. A suitable antioxidant may be added.

Nominal Content of Monoglycerides (%)			
	40	**60**	**90**
Monoglycerides	32.0–52.0	55.0–65.0	90.0–101.0
Diglycerides	30.0–50.0	15.0–35.0	<10.0
Triglycerides	5.0–20.0	2.0–10.0	<2.0

IDENTIFICATION

- **A. Thin-Layer Chromatographic Identification Test** ⟨**201**⟩
  Standard solution: 50 mg/mL of USP Glyceryl Monooleate 40% RS or USP Glyceryl Monooleate 90% RS in methylene chloride
  Sample solution: 50 mg/mL of Glyceryl Monooleate in methylene chloride
  Application volume: 10 µL
  Developing solvent system: Ether and hexane (7:3)
  Spray reagent: 0.1 mg/mL of rhodamine B in alcohol
  Analysis: Proceed as directed in the chapter. Spray with the *Spray reagent*, and locate the spots on the plate by examination under UV light at a wavelength of 365 nm.
  Acceptance criteria: The principal spot of the *Sample solution* corresponds in color, size, and R_F value to that of the *Standard solution*.
- **B.** It meets the requirements in *Specific Tests* for *Fats and Fixed Oils, Iodine Value* ⟨401⟩.

ASSAY

- **Procedure**
  Mobile phase: Tetrahydrofuran
  Sample solution: 40 mg/mL of Glyceryl Monooleate in tetrahydrofuran
  Chromatographic system
  (See *Chromatography* ⟨621⟩*, System Suitability*.)
  Mode: LC
  Detector: Refractive index
  Column: 7.5-mm × 60-cm; 5-µm 100-Å packing L21
  [Note—Two or three 7.5-mm × 30-cm L21 columns may be used in place of the one 60-cm column, provided that the system suitability requirements are met. The column temperature may be lowered to ambient temperature, although working at 40° provides stable separation conditions and ensures better sample solubility.]
  Temperature
  Column: 40°
  Detector: 40°
  Flow rate: 1 mL/min
  Injection size: 40 µL
  System suitability
  Sample: *Sample solution*
  [Note—The relative retention times for triglycerides, diglycerides, monoglycerides, and glycerin are about 0.76, 0.79, 0.85, and 1.0, respectively.]
  Suitability requirements
  Resolution: NLT 1.0 between the diglycerides and monoglycerides
  Relative standard deviation: NMT 2.0%, determined from the monoglycerides peak
  Analysis
  Sample: *Sample solution*
  Calculate the percentage of monoglycerides, diglycerides, and triglycerides in the portion of Glyceryl Monooleate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

  r_U = individual peak responses for the monoglycerides, diglycerides, and triglycerides, as appropriate
  r_T = sum of all the glyceride peak responses
  Acceptance criteria: See the table in the *Definition*.

IMPURITIES

- **Limit of Free Glycerin**
  Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solutions: Prepare four solutions by dissolving glycerin in tetrahydrofuran, and diluting each with tetrahydrofuran as necessary, to obtain solutions having known concentrations of 0.4, 1.0, 2.0, and 4.0 mg/mL.
Standard curve
Samples: *Standard solutions*
Plot: Record the chromatograms, and measure the responses for the glycerin peaks. Plot the glycerin peak responses obtained versus the concentration, in mg/mL, of glycerin in the *Standard solutions*.
Analysis
Sample: *Sample solution*
From the *Standard curve*, determine the glycerin concentration, in mg/mL, in the *Sample solution*.
Calculate the percentage of free glycerin in the portion of Glyceryl Monooleate taken:

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

C_S = concentration of glycerin in the *Sample solution* from the *Standard curve* (mg/mL)
C_U = concentration of Glyceryl Monooleate in the *Sample solution* (mg/mL)
Acceptance criteria: NMT 6.0%

SPECIFIC TESTS

- **FATS AND FIXED OILS,** *Acid Value* ⟨**401**⟩**:** NMT 6.0, determined on 1.0 g
- **FATS AND FIXED OILS,** *Iodine Value* ⟨**401**⟩**:** 65.0–95.0
- **FATS AND FIXED OILS,** *Peroxide Value* ⟨**401**⟩**:** NMT 12.0, determined on 2.0 g
- **FATS AND FIXED OILS,** *Saponification Value* ⟨**401**⟩**:** 150–175, determined on 2.0 g
- **FATS AND FIXED OILS,** *Fatty Acid Composition* ⟨**401**⟩**:** Glyceryl Monooleate exhibits the following composition profile of fatty acids (see *Table 1*), determined as directed in the chapter.

Table 1

Carbon-Chain Length	Number of Double Bonds	Percentage, NMT (%)
16	0	12.0
18	0	6.0
18	1	60.0
18	2	35.0
18	3	2.0
20	0	2.0
20	1	2.0

- **WATER DETERMINATION,** *Method I* ⟨**921**⟩**:** NMT 1.0%, using a mixture of methanol and methylene chloride (1:1) in place of methanol in the titration vessel
- **ARTICLES OF BOTANICAL ORIGIN,** *Total Ash* ⟨**561**⟩**:** NMT 0.1%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. No storage requirements specified.
- **LABELING:** The labeling indicates the nominal content of monoglycerides and the name and the concentration of any added antioxidant.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Glyceryl Monooleate 40% RS
  USP Glyceryl Monooleate 90% RS

Glyceryl Monostearate

Octadecanoic acid, monoester with 1,2,3-propanetriol; Monostearin [31566-31-1].

DEFINITION

Glyceryl Monostearate contains NLT 90.0% of monoglycerides of saturated fatty acids, chiefly glyceryl monostearate ($C_{21}H_{42}O_4$) and glyceryl monopalmitate ($C_{19}H_{38}O_4$). It may contain a suitable antioxidant.

ASSAY

- **PROCEDURE**
Mobile phase: Tetrahydrofuran
Sample solution: 8 mg/mL of Glyceryl Monostearate in tetrahydrofuran
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: Refractive index
Column: 7.5-mm × 60-cm; 5-µm 100-Å packing L21
Temperature: Column and detector temperatures are maintained at 40°.
[NOTE—Two or three 7.5-mm × 30-cm L21 columns may be used in place of the one 60-cm column, provided that system suitability requirements are met. The column temperature may be lowered to ambient temperature, although working at 40° provides stable separation conditions and ensures better sample solubility.]
Flow rate: 1 mL/min
Injection size: 40 µL
System suitability
Sample: *Sample solution*
[NOTE—The relative retention times for triglycerides, diglycerides, monoglycerides, and glycerin are 0.77, 0.81, 0.86, and 1.0, respectively.]
Suitability requirements
Relative standard deviation: NMT 2.0%, determined from the monoglycerides peak
Analysis
Sample: *Sample solution*
Calculate the percentage of monoglycerides in the portion of Glyceryl Monostearate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of the monoglycerides
r_T = sum of all the glyceride peak responses
Acceptance criteria: NLT 90.0% of monoglycerides of saturated fatty acids, chiefly $C_{21}H_{42}O_4$ and $C_{19}H_{38}O_4$

IMPURITIES

Inorganic Impurities
- **RESIDUE ON IGNITION** ⟨**281**⟩**:** NMT 0.5%
- **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 10 ppm

Organic Impurities
- **PROCEDURE: LIMIT OF FREE GLYCERIN**
Propionating reagent: Pyridine and propionic anhydride (1:2)
Internal standard solution: 0.2 mg/mL of tributyrin in chloroform
Standard solution: Transfer 15 mg of glycerin and 50 mg of tributyrin to a glass-stoppered, 25-mL conical flask. Add 3 mL of *Propionating reagent*, and heat at 75° for 30 min. Volatilize the reagents with the aid of a stream of nitrogen at room temperature, and add 12 mL of chloroform. Dilute 1 mL of this mixture with chloroform to 20 mL.
Sample solution: Transfer 50 mg of Glyceryl Monostearate to a glass-stoppered, 25-mL conical flask. Add 5 mL of *Internal standard solution* by pipet, and mix to dissolve. Immerse the flask in a water bath, maintained at a temperature between 45° and 50°, and volatilize the chloroform with the aid of a stream of nitrogen. Add 3 mL of *Propionating reagent*, and heat at 75° for 30 min. Volatilize the reagents with the aid of a stream of nitrogen at room temperature, and add 5 mL of chloroform.
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)