Designation: D 1983 – 90 (Reapproved 1995)ε1

An American National Standard

AMERICAN SOCIETY FOR TESTING AND MATERIALS
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Standard Test Method for Fatty Acid Composition by Gas-Liquid Chromatography of Methyl Esters[1]

This standard is issued under the fixed designation D 1983; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

This standard has been approved for use by agencies of the Department of Defense.

ε[1] NOTE—Unit of measurement statement added editorially in May 1995.

1. Scope

1.1 This test method establishes standard conditions for the separation and identification of methyl esters by gas-liquid chromatography.

1.2 This test method is applicable to animal and vegetable fatty acids and oils having 8 to 24 carbon atoms. The use of the polyester liquid phase facilitates the separation of both the saturated and various unsaturated fatty acid methyl esters on the chromatogram obtained.

1.3 The conditions specified in this test method are not suitable for determining epoxy and oxidized fatty acids nor to fatty acids that have been polymerized. See also Test Methods D 2800 and D 3457.

1.4 The values stated in SI units are to be regarded as the standard. The values given in parentheses are for information only.

1.5 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*

D 2800 Test Method for Preparation of Methyl Esters from Oils for Determination of Fatty Acid Composition by Gas-Liquid Chromatography[2]

D 3457 Test Method for Preparation of Methyl Esters from Fatty Acids for Determination of Fatty Acid Composition by Gas-Liquid Chromatography[2]

3. Significance and Use

3.1 This test method provides a means for identifying vegetable oils as to type by comparing to known standards. It can also be used to detect adulteration of one vegetable oil by another.

3.2 The amount or the proportion of one specific acid can be used for specification purposes, for example, the amount of linolenic acid in linseed oil or the percent of linoleic acid in sunflower oil.

3.3 By measuring the amount of total eluted acids by use of an internal standard, an estimation may be made of the amount of polymerization of the fatty acids present in a polymerized oil.

4. Apparatus

4.1 *Gas Chromatographic Instrument* having the following minimal characteristics:

4.1.1 *Column Oven*, operated at a constant temperature between 190 and 210°C.

4.1.2 *Sample Inlet Port*, with the heater characteristics necessary for operation at 60°C higher than the maximum necessary column oven temperature.

4.1.3 *Detector*, of the flame ionization or thermal conductivity type. If separately thermostatted, it should be maintained at column temperature or hotter.

4.1.4 *Column*, 1.5 to 3.0 m (5 to 10 ft) long, 6.4 mm (¼ in.) in outside diameter, made of glass, stainless steel, copper, or aluminum packed with 20 weight % of polydiethylene glycol succinate polyester (DEGS) liquid phase on 80 to 100 mesh acid washed calcined diatomaceous earth.[3]

4.1.5 *Recorder*, 0 to 1-mV range, 1-s full-scale deflection with a chart speed of 13 to 25 mm (½ to 1 in.)/min, and an attenuator switch to change the recorder range as required; the recorder should be equipped with an integrator if possible.

4.1.6 *Helium Carrier Gas*, pure.

[1] This method is under the jurisdiction of ASTM Committee D-1 on Paint and Related Coatings, Materials, and Applications, and is the direct responsibility of Subcommittee D01.32 on Drying Oils.

Current edition approved May 25, 1990. Published July 1990. Originally published as D 1983 – 64 T. Last previous edition D 1983 – 75(1980)ε1.

[2] *Annual Book of ASTM Standards*, Vol 06.03.

[3] Chromosorbs W and P manufactured by Manville Sales Corp., available from gas chromatography suppliers, have been found satisfactory for this purpose.

4.2 *Syringe for Injecting Specimens*, fixed needle, 10-µL capacity or equivalent with a known and reproducible volume.

4.3 *Electronic or Mechanical Integrator*.

5. Reagents

5.1 *Standard Fatty Acid Methyl Ester* containing approximately equal quantities of oleic and stearic methyl esters, for optimizing operating conditions.

6. Preparation of Apparatus

6.1 Start the flow of helium gas through the apparatus and adjust the inlet port, column, and detector, if individually thermostatted, to their operating conditions as given in 4.1. Record a base line to check for stability of the instrument. Normally a new column with the DEGS liquid phase must be preconditioned by maintaining it at its operating temperature with helium flowing through it for 24 h or until the recorder base line is stable at the most sensitive attenuation setting to be used.

NOTE 1—At no time should the detector filament current (thermal conductance (TC) detector) be turned on when helium gas is not flowing through the detector.

6.2 The proper gas flow rate should permit elution of linolenic and shorter chain methyl esters in 30 min or less. The inlet pressure and gas flow necessary to accomplish this varies between columns and instruments used but are relatively constant for a single apparatus. It should not be necessary to exceed 280 kPa (40 psi) for the gas pressure at the inlet of the flow control capillaries. A constant gas flow should be maintained throughout the duration of an analysis to maintain linearity of signal response. Polyester stationary phases are very susceptible to oxygen damage and hydrolysis. The use of gas purifiers and oxygen removers is recommended for the carrier gas.

6.3 Take up 0.5 to 3 µL of fatty acid ester standard (see 5.1) into the syringe. Wipe the needle tip, pierce the septum of the sample inlet port, quickly discharge the specimen, and withdraw the needle immediately. Note on the recorder chart the small peak caused by air which marks the sample introduction reference point. This will be followed immediately by the ether solvent peak if there is some residual solvent left in the sample. The specimen size must be adjusted so that the major peak does not exceed the linearity range of the detector. Check manufacturer's specification.

NOTE 2—The specimen must be discharged rapidly so that uniform flash vaporization occurs or the phenomenon of "tailing" may occur which precludes the possibility of sharp separations.

6.4 Having determined the optimum conditions, inject a second specimen of the methyl ester standard (see 5.1) and watch the recorder pen to see that the peaks do not go off scale. Change the setting of the attenuator if necessary to keep the peaks on the chart paper. Note the attenuation on the chart at the point that changes are made.

6.5 Determine the instrument and column performance by noting the separation of the oleate and stearate peaks (see 6.4). This separation is expressed as peak resolution, *R*, as follows:

$$R = 2Y/(S + O)$$

where:

Y = distance between the peak maxima for stearate and oleate,
S = base width of the stearate peak, and
O = base width of the oleate peak.

NOTE 3—These values should be determined on a sample containing approximately equal quantities of oleate and stearate esters using a specimen size such that these peaks are 25 to 50 % of the chart width. If the peak resolution is equal to or greater than 1.0 the column and instrument are in satisfactory condition. All columns when used will show a gradual loss in peak resolution. When the value becomes less than 1.0, a new column should be installed.

7. Calibration

7.1 Determine calibration factors to correct for nonlinearity of instrument response due to molecular weight differences. In most cases the standard mixtures are not made up with exactly the same weights of each ester. The units of area per weight percent must be calculated by dividing the area of each peak in the standard mixture by its weight percent. Then relative response values can be calculated by dividing this number by the units of area per weight percent obtained for the palmitate. Compare the calculated values with those listed in Table 1; make sure that they are nearly the same.

NOTE 4—Careful workers in the field have reported variations in relative response when the ratio of one fatty acid to another in a standard mixture is greatly changed. The magnitude of the variation is generally considered close enough to the precision of the test method that it does not appear practical to use these small corrections in routine work. However, for precise analysis the corrections may be desirable.

8. Procedure

8.1 Using the same condition as for the standard record the chromatogram of the fatty acid methyl esters prepared in accordance with Test Methods D 2800 or D 3457 using attenuation settings that provide peak heights of principal components between 15 and 85 % of full scale. Observe the usual cautions described in 6 through 6.5 in the chromatogramming of the standard methyl ester.

NOTE 5—High-boiling constituents, polymers, unsaponifiables, and rosin acids that may be present in appreciable amounts in certain types of fatty acids are likely not to be eluted from the column and thus cause errors. By using an internal standard, these errors can not only be eliminated, but if the interest is in only certain of the fatty acids present, these can readily be calculated on the original sample basis without measuring all the peaks.

8.2 After all the peaks have been traced and the pen has returned to the base line, remove the chart for identification.

8.3 Identify the peaks by relative position on the chart.

TABLE 1 Relative Retention and Relative Response Values for Methyl Esters of Fatty Acids

Ester	Relative Retention	Relative Response
Myristate	0.56	1.03
Palmitate	1.00	1.00
Margarate	1.32	0.99
Stearate	1.70	0.97
Oleate	1.94	0.95
Linoleate	2.31	0.92
Linolenate	3.00	0.90