Designation: D6584 – 13

Standard Test Method for Determination of Total Monoglycerides, Total Diglycerides, Total Triglycerides, and Free and Total Glycerin in B-100 Biodiesel Methyl Esters by Gas Chromatography[1]

This standard is issued under the fixed designation D6584; 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.

1. Scope*

1.1 This test method covers the quantitative determination of total monoglyceride, total diglyceride, total triglyceride, and free and total glycerin in B-100 methyl esters by gas chromatography. The range of quantitation for monoglyceride is 0.009 to 0.77860 mass %, for diglyceride is 0.092353 to 0.54475 mass %, and for triglyceride is 0.00092857 to 1.3881 mass %. The range of quantitation for free glycerin is 0.0005714 to 0.019533 mass % and for total glycerin from 0.0090714 to 0.42767 mass %. This procedure is not applicable to vegetable oil methyl esters obtained from lauric oils, such as coconut oil and palm kernel oil.

1.2 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.3 *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:*[2]

D4307 Practice for Preparation of Liquid Blends for Use as Analytical Standards

E355 Practice for Gas Chromatography Terms and Relationships

E594 Practice for Testing Flame Ionization Detectors Used in Gas or Supercritical Fluid Chromatography

3. Terminology

3.1 *Definitions:*

3.1.1 *biodiesel (B-100), n*—fuel comprised of mono-alkyl esters of long chain fatty acids derived from vegetable oils or animal fats.

3.1.2 *bonded glycerin, n*—glycerin portion of the mono-, di-, and triglyceride molecules.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *total glycerin, n*—sum of free and bonded glycerin.

3.3 This test method makes reference to many common gas chromatographic procedures, terms, and relationships. Detailed definitions can be found in Practices E355 and E594.

4. Summary of Test Method

4.1 The sample is analyzed by gas chromatography, after silyating with N-methyl-N-trimethylsilyltrifluoracetamide (MSTFA). Calibration is achieved by the use of two internal standards and four reference materials. Mono-, di-, and triglyceride are determined by comparing to monoolein, diolein, and triolein standards respectively. Average conversion factors are applied to mono-, di-, and triglycerides to calculate the bonded glycerin content of the sample.

5. Significance and Use

5.1 Free and bonded glycerin content reflects the quality of biodiesel. A high content of free glycerin may cause problems during storage, or in the fuel system, due to separation of the glycerin. A high total glycerin content can lead to injector fouling and may also contribute to the formation of deposits at injection nozzles, pistons, and valves.

6. Apparatus

6.1 *Chromatographic System*—See Practice E355 for specific designations and definitions.

6.1.1 *Gas Chromatograph (GC)*—The system must be capable of operating at the conditions given in Table 1.

6.1.2 *Column,* open tubular column with a 5 % phenylpolydimethylsiloxane bonded and cross linked phase internal coating. The column should have an upper temperature limit of at least 400°C. Columns, either 10 m or 15 m in length, with a 0.32 mm internal diameter, and a 0.1 µm film thickness have been found satisfactory. Any column with better or equivalent

[1] This test method is under the jurisdiction of ASTM Committee D02 on Petroleum Products, Liquid Fuels, and Lubricants and is the direct responsibility of D02.04.0L on Gas Chromatography Methods.

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

***A Summary of Changes section appears at the end of this standard**

TABLE 1 Operating Conditions

	Injector	
Cool on column injection		
Sample size	1 µL	
	Column Temperature Program	
Initial temperature	50°C	hold 1 min
Rate 1	15°C / min to 180°C	
Rate 2	7°C / min to 230°C	
Rate 3	30°C / min 380°C	hold 10 min
	Defector	
Type	Flame ionization	
Temperature	380°C	
	Carrier Gas	
Type	Hydrogen or helium	measured at 50°C
Flow rate	3 mL/min	

TABLE 2 Stock Solutions

Compound	CAS No.	Approximate Mass (mg)	Volumetric Flask Size (mL)
Glycerin	56-81-5	25	50
1-Mono [*cis*-9-octadecenoyl]-*rac*-glycerol (monoolein)	111-03-5	50	10
1,3-Di [*cis*-octadecenoyl]glycerol (diolein)	2465-32-9	50	10
1,2,3-Tri [*cis*-octadecenoyl]glycerol (triolein)	122-32-7	50	10
(S) - (-) -1,2,4-Butanetriol - (Internal Standard 1)	42890-76-6	25	25
1,2,3-Tridecanolylglycerol (tricaprin) - (Internal Standard 2)	621-71-6	80	10

chromatographic efficiency and selectivity can be used. It is recommended that a 2 to 5 metre 0.53 mm high temperature guard column be installed from the injector to the analytical column. This allows the use of autoinjectors and also increases column life.

6.2 *Electronic Data Acquisition System:*

6.2.1 *Integrator or Computer,* capable of providing real time graphic and digital presentation of the chromatographic data is recommended for use. Peak areas and retention times shall be measured by computer or electronic integration.

6.2.2 This device must be capable of performing multilevel internal-standard-type calibrations and be able to calculate the correlation coefficient (r^2) and internal standard calculations for each data set.

7. Reagents and Materials

7.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society where such specifications are available.[3] Other grades may be used provided it is first ascertained that the reagent is of sufficient purity to permit its use without lessening the accuracy of the determination.

7.2 *n-Heptane,* reagent grade.

7.3 *N-Methyl-N-trimethylsilyltrifluoroacetamide (MSTFA),* reagent grade.

7.4 *Pyridine,* reagent grade.

7.5 *Carrier Gas,* hydrogen or helium of high purity. Additional purification is recommended by the use of molecular sieves or other suitable agents to remove water, oxygen, and hydrocarbons. Available pressure must be sufficient to ensure a constant carrier gas flow rate.

7.6 *Microlitre Syringes,* 100 µL and 250 µL capacity.

7.7 *Screw Cap Vials,* with polytetrafluoroethylene (PTFE)-faced septa, 10 mL capacity.

8. Preparation of Apparatus

8.1 Install and condition the column in accordance with manufacturer or supplier's instructions. After conditioning, attach column outlet to flame ionization detector inlet and check for leaks throughout the system. If leaks are found, tighten or replace fittings and recheck for leaks before proceeding.

9. Calibration and Standardization

9.1 *Preparation of Calibration Standards*—Prepare standards using fresh compounds listed in Table 2 according to Practice D4307. Weigh the components directly into the volumetric flasks specified and record the mass to the nearest 0.1 mg. Dilute the volumetric flasks to mark with pyridine. Store the calibration standards in a refrigerator when not in use.

9.2 *Standard Solutions*—Prepare the five standard solutions in Table 3 by transferring the specified volumes by means of microlitre syringes to 10 mL septa vials. Add to each of the five standard solutions 100 µL of MSTFA. Close the vial and shake. Allow the vial to stand for 15 to 20 min at room temperature. Add approximately 8 mL n-Heptane to the vial and shake.

9.3 *Chromatographic Analysis*—If using an automatic sampler, transfer an aliquot of the solution into a glass GC vial and seal with a TFE-fluorocarbonlined cap.

9.4 *Standardization*—Analyze the calibration standards under the same operating conditions as the sample solutions. Inject 1 µL of the reaction mixture into the cool on-column injection port and start the analysis. Obtain a chromatogram and peak integration report. For each reference substance, determine the response ratio (rsp_i) and amount ratio (amt_i) for each component using Eq 1 and 2.

$$rsp_i = (A_i/A_s) \quad (1)$$

where:

A_i = area of reference substance, and
A_s = area of internal standard.

$$amt_i = (W_i/W_s) \quad (2)$$

where:

W_i = mass of reference substance, and
W_s = mass of internal standard.

9.4.1 Prepare a calibration curve for each reference component by plotting the response ratios (rsp_i), as the y-axis, versus the amount ratios (amt_i), as the x-axis.

[3] *Reagent Chemicals, American Chemical Society Specifications*, American Chemical Society, Washington, DC. For Suggestions on the testing of reagents not listed by the American Chemical Society, see *Annual Standards for Laboratory Chemicals,* BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary,* U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

TABLE 3 Standard Solutions

Standard Solution Number	1	2	3	4	5
µL of glycerin stock solution	10	30	50	70	100
µL of monoolein stock solution	20	50	100	150	200
µL of diolein stock solution	10	20	40	70	100
µL of triolein stock solution	10	20	40	70	100
µL of butanetriol stock solution	100	100	100	100	100
µL of tricaprin stock solution	100	100	100	100	100

9.5 Calculate the correlation coefficient r^2 value for each reference component in the calibration set using Eq 3. The r^2 value should be at least 0.99 or greater. If the above criteria for r^2 are not met, rerun the calibration or check instrument parameters and hardware.

$$r^2 = \frac{\left(\sum xy\right)^2}{\left(\sum x^2\right)\left(\sum y^2\right)} \tag{3}$$

where:

$$x = X_i - \bar{x} \tag{4}$$

$$y = Y_i - \bar{y} \tag{5}$$

and:

X_i = amt_i ratio data point,
$\bar{x}$ = average values for all amt_i data points
Y_i = corresponding rsp_i data points,
$\bar{y}$ = average values for all rsp_i data points.

9.6 *Calibration Functions*—For each reference calibration functions are calculated in the form:

$$\frac{A_x}{A_{is}} = \left[a_x\left(\frac{W_x}{W_{is}}\right)\right] + b_x \tag{6}$$

where:

W_x = mass of reference substance, mg,
W_{is} = mass of internal standard, mg,
A_x = peak area of reference substance,
A_{is} = peak area of internal standard,
a_x = slope of the calibration function, and
b_x = intercept of the calibration function.

10. Procedure

10.1 Set the instrument operating variables to the values specified in Table 1. Weigh to the nearest 0.1 mg approximately 100 mg of sample directly into a 10 mL septa vial. Using microlitre syringes, add exactly 100 µL of each internal standard and MSTFA. Shake the vials, and allow to set for 15 to 20 min at room temperature. Add approximately 8 mL of n-Heptane to the vial and shake.

10.2 Inject 1 µL of the reaction mixture into the cool on-column injection port and start the analysis. Obtain a chromatogram and peak integration report.

10.3 *Peak Identification*—Identify peaks by comparison of retention times to the standards. For identification of additional peaks, use the relative retention times given in Table 4 and the reference chromatograms given in Fig. 1. Mono-, di-, and triglycerides are separated according to carbon numbers (CN).

10.4 Monoglyceride consists of the four overlapping peaks with relative retention times (RRT) of 0.76 and 0.83 to 0.86 with respect to the internal standard tricaprin. A pair of peaks, methyl esters with a carbon number of 24, may appear with RRT of 0.80 to 0.82, and should not be included in the calculation of monoglyceride.

TABLE 4 Approximate Relative Retention Times

Component	Use Internal Standard	Relative Retention Time
Glycerin	1	0.85
1,2,4 Butanetriol Internal Standard 1		1.00
Monopalmitin	2	0.76
Monoolein, monolinolein monolinolenin, and monostearin	2	0.83-0.86
Tricaprin Internal Standard 2		1.00
Diglycerides	2	1.05-1.09
Triglycerides	2	1.16-1.31

10.5 Diglyceride is also primarily separated according to carbon number, but due to varying double bonds in the molecules, baseline resolution of the peaks does not occur. The grouping of 3 to 4 peaks with RRT of 1.05 to 1.09 (CN 34, 36, and 38) shall be attributed to diglyceride. Carbon number also separates triglyceride. Peaks with RRT of 1.16 to 1.31 (CN 52, 54, 56, and 58) should be included in the calculation.

11. Calculation and Report

11.1 After identifying the peaks, measure the areas of the peaks identified as glycerin, mono-, di-, and triglyceride. Using the slope and y-intercept of the calibration functions, calculate the mass of each as follows:

11.1.1 *Glycerin:*

$$G = \left[\frac{W_{is1}}{a_g}\right]\left(\left[\frac{A_g}{A_{is}}\right] - b_g\right)\left[\frac{100}{W}\right] \tag{7}$$

where:

G = mass percentage of glycerin in sample,
A_g = peak area of glycerin,
A_{is1} = peak area of Internal Standard 1,
W_{is1} = weight of Internal Standard 1, mg,
W = weight of sample, mg,
a_g = slope of the calibration function,
b_g = intercept of the calibration function.

11.1.2 *Individual Glyceride:*

$$Gl_j = \left[\frac{W_{is2}}{a_{ol}}\right]\left(\left[\frac{A_{glj}}{A_{is2}}\right] - b_{o1}\right)\left[\frac{100}{W}\right] \tag{8}$$

where:

Gl_j = mass percentage of individual glyceride in sample,
A_{glj} = peak area of individual glyceride,
$A_{is\,2}$ = peak area of Internal Standard 2,
W_{is2} = weight of Internal Standard 2, mg,
W = weight of sample, mg,
a_{ol} = slope of the calibration function for mono, di-, or triolein, and
b_{ol} = intercept of the calibration function for mono, di, or triolein.

11.1.3 *Calculation of Total Glycerin:*

$$\text{total glycerin} = \text{free glycerin} + \text{bound glycerin} \tag{9}$$

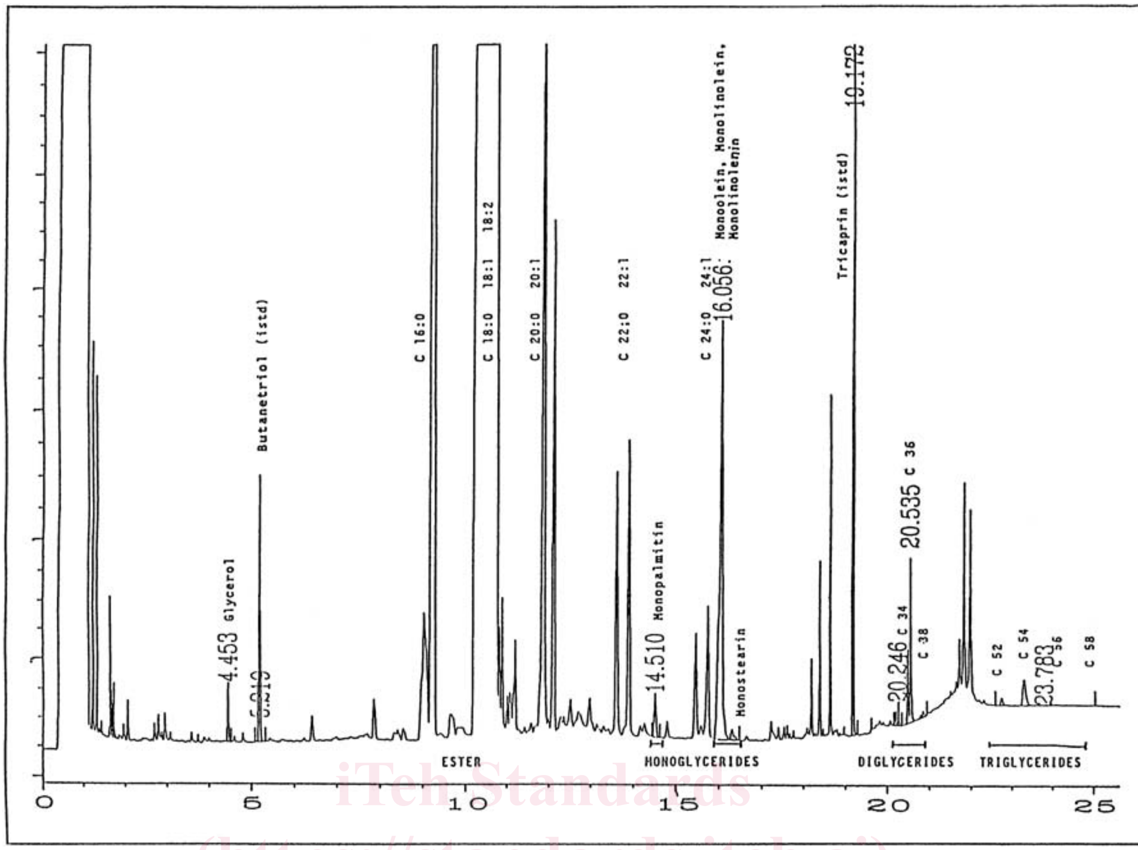

FIG. 1 Reference Chromatograms

where:

free glycerin = glycerin determined in Eq 7,
bound glycerin = $\sum$ (Gl$_M$, Gl$_D$, Gl$_T$)

where:

Gl_M = 0.2591 × $\sum$ monoglyceride, mass % determined in Eq 8,
Gl_D = 0.1488 × $\sum$ diglyceride, mass % determined in Eq 8, and
Gl_T = 0.1044 × $\sum$ triglyceride, mass % determined in Eq 8.

11.2 Report the total monoglyceride, total diglyceride, total triglyceride, and free and total glycerin to the nearest 0.001 mass %.

12. Precision and Bias

12.1 The precision of this procedure, as determined by statistical examination of the 2012 interlaboratory test results,[4] obtained from 11 laboratories on 14 B-100 biodiesel samples from a variety of sources, is as follows:

12.1.1 *Repeatability*—The difference between successive results obtained by the same operator with the same apparatus under constant operating conditions on identical test material, would in the long run, in the normal and correct operation of the test method, exceed the following values in on case in twenty.

12.1.1.1 *Total Glycerin Repeatability:*

$$r = 0.76E^{-01} * TG^{0.73} \quad (10)$$

where:

TG = the calculated result for total glycerin expressed as a mass % of the glycerin content in the sample, and
r = repeatability.

12.1.1.2 *Free Glycerin Repeatability:*

$$r = 0.195E^{-01} * FG + 0.0001^{0.27} \quad (11)$$

where:

FG = the calculated result for free glycerin expressed as a mass % of the glycerin content in the sample, and
r = repeatability.

12.1.1.3 *Total Monoglyceride Repeatability:*

$$r = 0.78E^{-01} * M^{0.62} \quad (12)$$

where:

M = total monoglyceride concentration in mass %, and
r = repeatability.

12.1.1.4 *Total Diglyceride Repeatability:*

$$r = 0.344 * D^{0.93} \quad (13)$$

where:

D = total diglyceride concentration in mass %, and
r = repeatability.

[4] Supporting data have been filed at ASTM International Headquarters and may be obtained by requesting Research Report RR:D02-1756.