



## Test Method for Determination of Free and Total Glycerin in B-100 Biodiesel Methyl Esters By Gas Chromatography<sup>1</sup>

This standard is issued under the fixed designation D 6584; 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 ( $\epsilon$ ) indicates an editorial change since the last revision or reapproval.

### 1. Scope

1.1 This test method provides for the quantitative determination of free and total glycerin in B-100 methyl esters by gas chromatography. The range of detection for free glycerin is 0.005 to 0.05 mass %, and total glycerin from 0.05 to 0.5 mass %. This procedure is not applicable to vegetable oil methyl esters obtained from lauric oils, such as coconut oil and palmkernel oil.

1.2 The values stated in SI units are to be regarded as the 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:

D 4307 Practice for Preparation of Liquid Blends for Use as Analytical Standards<sup>2</sup>

E 355 Practice for Gas Chromatography Terms and Relationships<sup>3</sup>

E 594 Practice for Testing Flame Ionization Detectors Used in Gas or Supercritical Fluid Chromatography<sup>3</sup>

### 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*—is the glycerin portion of the mono-, di-, and triglyceride molecules.

3.1.3 *total glycerin, n*—is the sum of free and bonded glycerin.

3.2 This test method makes reference to many common gas chromatographic procedures, terms, and relationships. Detailed definitions can be found in Practices E 355 and E 594.

### 4. Summary of Test Method

4.1 The sample is analyzed by gas chromatography, after silylating with N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA). Calibration is achieved by the use of two internal standards and four reference materials. Mono-, di-, and triglycerides are determined by comparing to monoolein, diolein, and triolein standards respectively. Average conversion factors are applied to the 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 D 355 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  $\mu\text{m}$  film thickness have been found satisfactory. Any column with better or equivalent 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.

<sup>1</sup> This test method is under the jurisdiction of ASTM Committee D02 on Petroleum Products and Lubricants and is the direct responsibility of D02.04 on Hydrocarbon Analysis.

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<sup>2</sup> *Annual Book of ASTM Standards*, Vol 05.02.

<sup>3</sup> *Annual Book of ASTM Standards*, Vol 14.02.

**TABLE 1 Operating Conditions**

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

## 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.<sup>4</sup> 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  $\mu$ l and 250  $\mu$ l 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 D 4307. 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.

<sup>4</sup> 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 *Analar 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 2 Stock Solutions**

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

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  $\mu$ L 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*:

9.5 Analyze the calibration standards under the same operating conditions as the sample solutions. Inject 1  $\mu$ L 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.

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.

9.6 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.

**TABLE 3 Standard Solutions**

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