



Test Method for Determination of Free and Total Glycerin in B-100 Biodiesel Methyl Esters By Gas Chromatography¹

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 (ϵ) 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 Standards²

E 355 Practice for Gas Chromatography Terms and Relationships³

E 594 Practice for Testing Flame Ionization Detectors Used in Gas or Supercritical Fluid Chromatography³

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 μm 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.

¹ 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.

Current edition approved Aug. 10, 2000. Published September 2000.

² *Annual Book of ASTM Standards*, Vol 05.02.

³ *Annual Book of ASTM Standards*, Vol 14.02.

TABLE 1 Operating Conditions

Injector		
Cool on column injection		
Sample size	1 μ L	
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
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.⁴ 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 μ L and 250 μ 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.

⁴ 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 μ 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 μ 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
μ L of glycerin stock solution	10	30	50	70	100
μ L of monoolein stock solution	20	50	100	150	200
μ L of diolein stock solution	10	20	40	70	100
μ L of triolein stock solution	10	20	40	70	100
μ L of butanetriol stock solution	100	100	100	100	100
μ L of tricaprin stock solution	100	100	100	100	100

$$r^2 = \frac{[\sum xy^2]}{[\sum x^2][\sum y^2]} \quad (3)$$

where:

$$\bar{x} = X_i - \bar{x} \quad (4)$$

$$\bar{y} = Y_i - \bar{y} \quad (5)$$

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.

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

$$W_x/W_{is} = a_x \times (A_x/A_{is}) + b_x \quad (6)$$

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 μ L 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 μ L 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. The mono-, di, and triglycerides are separated according to carbon numbers (CN).

10.4 Monoglycerides consist 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 monoglycerides.

TABLE 4 Approximate Relative Retention Times

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

10.5 Diglycerides are 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 diglycerides. Carbon number also separates triglycerides. 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 triglycerides. Using the slope and y-intercept of the calibration functions, calculate the mass of each as follows:

11.1.1 Glycerin:

$$G = (a_g \times A_g/A_{is1} + b_g) \times W_{is1} \times 100/W \quad (7)$$

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 Glycerides:

$$Gl_i = (a_o \times A_{gli}/A_{is2} + b_{o1}) \times W_{is2} \times 100/W \quad (8)$$

where:

Gl_i = mass percentage of individual glycerides in sample,

A_{gli} = peak area of individual glyceride,

A_{is2} = 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:

$$\text{total glycerin} = \text{free glycerin} + \text{bound glycerin} \quad (9)$$

where:

free glycerin = glycerin determined in Eq 7,

bound glycerin = $\sum (Gl_M, Gl_D, Gl_T)$

where:

Gl_M = $0.2591 \times \sum$ monoglyceride, mass % determined in Eq 8,

Gl_D = $0.1488 \times \sum$ diglyceride, mass % determined in Eq 8, and

Gl_T = $0.1044 \times \sum$ triglyceride, mass % determined in Eq 8.

11.2 Report the free and total glycerin to the nearest 0.001 mass %.

12. Precision and Bias

12.1 It is not feasible to provide a complete precision statement covering repeatability and reproducibility for this test method at this time since a sufficient quantity of repeat tests and samples under the required ASTM protocol are not

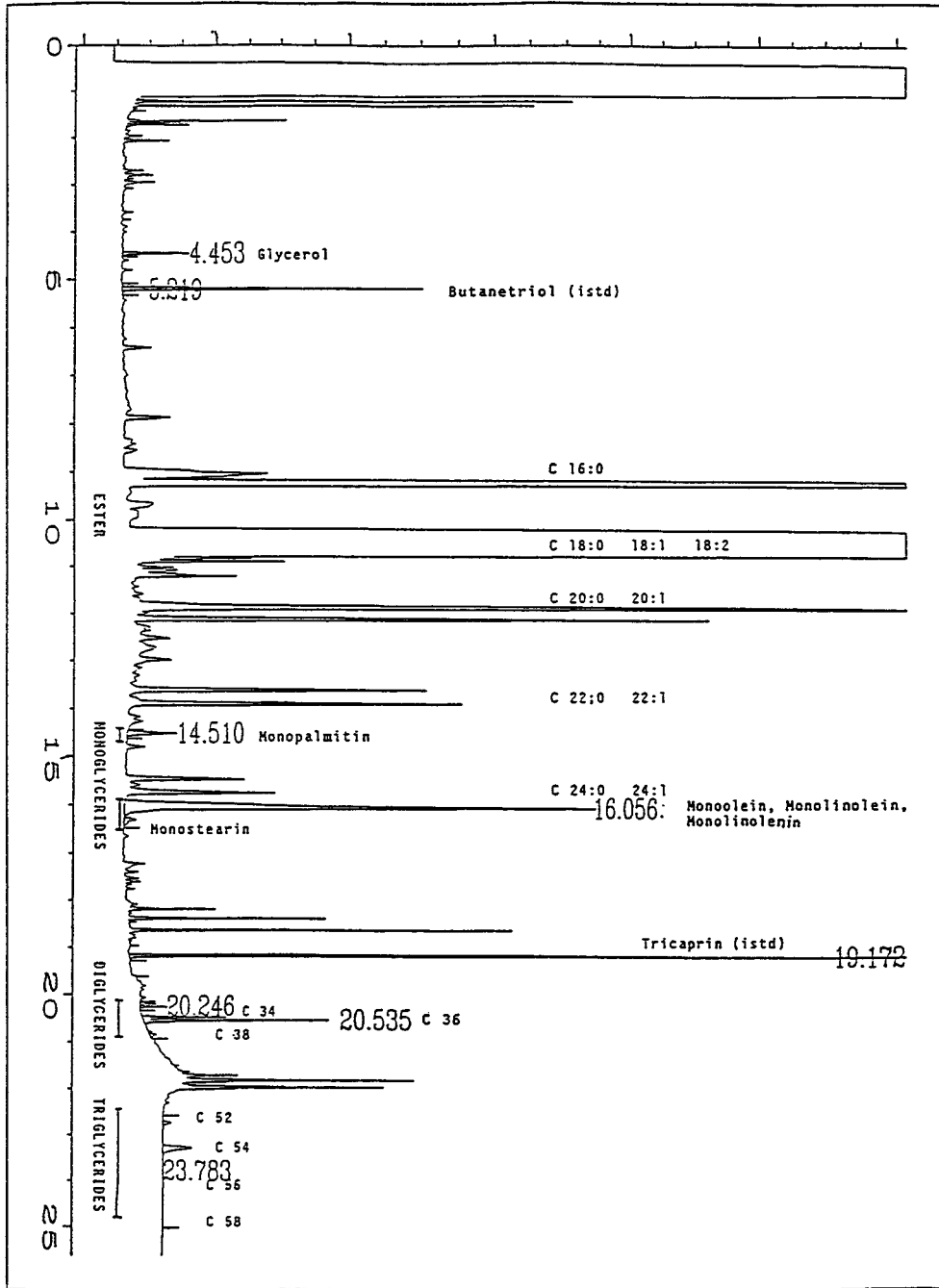


FIG. 1 Reference Chromatograms

available. This information is being determined and will be available on or before May 31, 2004.

12.2 *Repeatability*—As determined by a single laboratory, the difference between successive test results obtained by the same operator with the same apparatus under constant operating conditions on identical test materials would exceed the following values in only one case in twenty. See Table 5.

12.3 *Bias*—Since there is no accepted reference material suitable for determining bias for the procedure in this test method, bias can not be determined.

TABLE 5 Repeatability Estimates for Free and Total Glycerin Components

Component	Repeatability, mass %
Free glycerin	0.001
Monoglycerides	0.021
Diglycerides	0.021
Triglycerides	0.032
Bound glycerin	0.008
Total glycerin	0.009

13. Keywords

13.1 biodiesel; free glycerin; gas chromatography; methyl esters; total glycerin

ASTM International takes no position respecting the validity of any patent rights asserted in connection with any item mentioned in this standard. Users of this standard are expressly advised that determination of the validity of any such patent rights, and the risk of infringement of such rights, are entirely their own responsibility.

This standard is subject to revision at any time by the responsible technical committee and must be reviewed every five years and if not revised, either reapproved or withdrawn. Your comments are invited either for revision of this standard or for additional standards and should be addressed to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee, which you may attend. If you feel that your comments have not received a fair hearing you should make your views known to the ASTM Committee on Standards, at the address shown below.

This standard is copyrighted by ASTM International, 100 Barr Harbor Drive, PO Box C700, West Conshohocken, PA 19428-2959, United States. Individual reprints (single or multiple copies) of this standard may be obtained by contacting ASTM at the above address or at 610-832-9585 (phone), 610-832-9555 (fax), or service@astm.org (e-mail); or through the ASTM website (www.astm.org).