Designation: D6584 – 17

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 % by mass, for diglyceride is 0.092353 % to 0.54475 % by mass, and for triglyceride is 0.00092857 % to 1.3881 % by mass. The range of quantitation for free glycerin is 0.0005714 % to 0.019533 % by mass and for total glycerin from 0.0090714 % to 0.42767 % by 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.*

1.4 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

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.

[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

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 m to 5 m-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 min 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.

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

[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

where:

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

$$amt_i = (W_i/W_s) \tag{2}$$

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.

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.

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

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 min to 20 min at room temperature. Add approximately 8 mL of n-Heptane to the vial and shake.

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

10.2 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.

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,

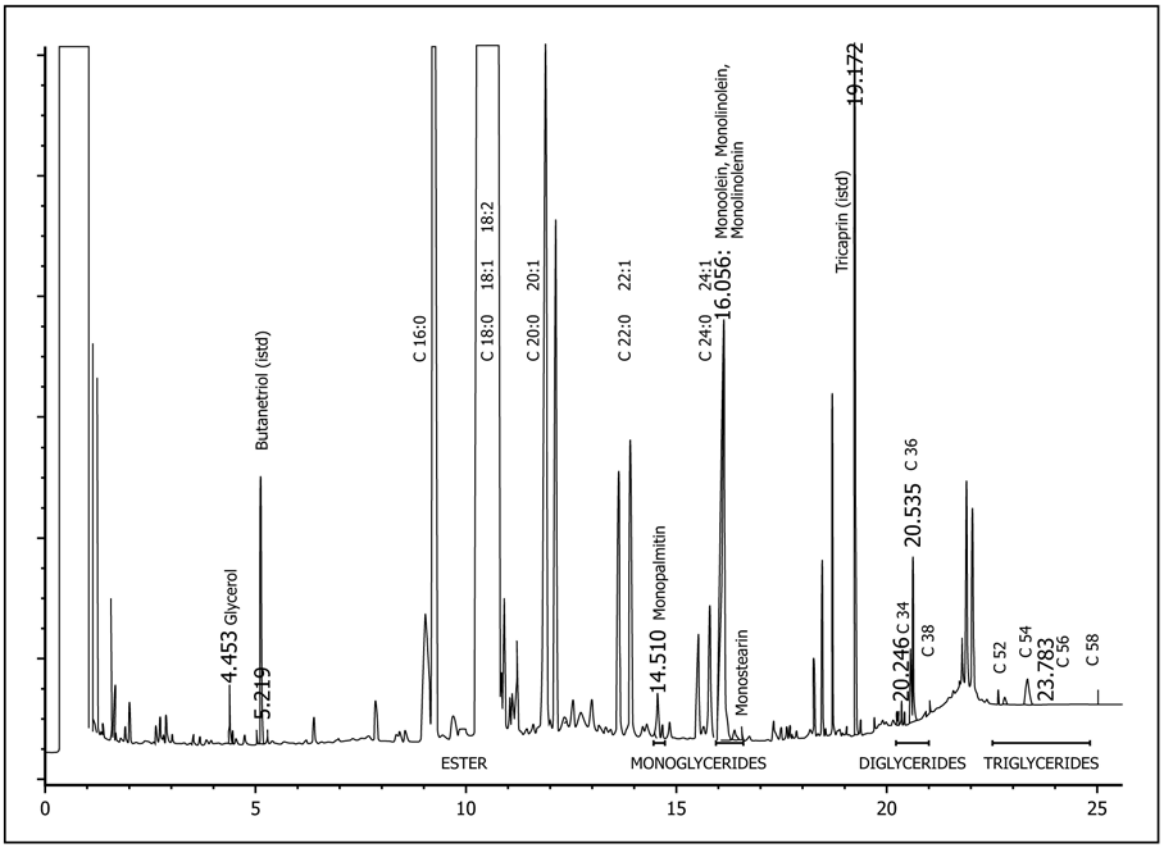

FIG. 1 Reference Chromatograms

A_{glj} = 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:*

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

where:

free glycerin = glycerin determined in Eq 7,
bonded 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 % by 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:*

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

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:*

[4] Supporting data have been filed at ASTM International Headquarters and may be obtained by requesting Research Report RR:D02-1756. Contact ASTM Customer Service at service@astm.org.

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

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:*

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

where:

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

12.1.1.4 *Total Diglyceride Repeatability:*

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

where:

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

12.1.1.5 *Total Triglyceride Repeatability:*

$$r = 0.12\cdot T^{0.687} \quad (14)$$

where:

T = total triglyceride concentration in mass %, and
r = repeatability.

12.1.2 *Reproducibility*—The difference between two single and independent results, obtained by different operators working in different laboratories on identical material, would in the long run, in the normal and correct operation of the test method, exceed the following values only in one case in twenty.

12.1.2.1 *Total Glycerin Reproducibility:*

$$R = 0.29\cdot TG^{0.73} \quad (15)$$

where:

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

12.1.2.2 *Free Glycerin Reproducibility:*

$$R = 0.246E^{-01}\cdot(FG+0.0001)^{0.27} \quad (16)$$

where:

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

12.1.2.3 *Total Monoglyceride Reproducibility:*

$$R = 0.46\cdot M^{0.62} \quad (17)$$

where:

M = total monoglyceride concentration in mass %, and
R = reproducibility.

12.1.2.4 *Total Diglyceride Reproducibility:*

$$R = 0.784\cdot D^{0.93} \quad (18)$$

where:

D = total diglyceride concentration in mass %, and
R = reproducibility.

12.1.2.5 *Total Triglyceride Reproducibility:*

$$R = 1.08\cdot T^{0.687} \quad (19)$$

where:

T = total triglyceride concentration in mass %, and
R = reproducibility.

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

13. Keywords

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

APPENDIX

(Nonmandatory Information)

X1. REFERENCE MIX AND EXAMPLE CHROMATOGRAMS FOR IMPROVED PEAK IDENTIFICATION

X1.1 Summary

X1.1.1 The variations in feedstock and transesterification methods employed in manufacturing B100 can result in variations in chromatograms of various samples, which can make proper identification of the monoglycerides, diglycerides and triglycerides difficult. Paragraph 10.3 details peak identification through comparison of retention times to internal standards—the mono-, di, and triglycerides are separated according to carbon numbers (CN). This appendix presents data to aid in that identification. This in no way purports to cover all B100 samples or feedstocks, but is added as a reference.

X1.1.2 Variations in the chromatographic system, column type and column age all contribute to successful separation of the glycerides in B100. Section 6 allows for the use of different column configurations and a wide variety of systems have been found to be suitable for this method. The example chromatograms shown in this section were all collected using a high temperature column (maximum temperature of 430 °C) with 5 % phenylpolydimethylsiloxane bonded and cross-linked phase of internal coating of 12 m length, with a 0.53 mm internal diameter with a 0.16 µm film thickness. A 2 m, with a 0.53 mm internal diameter high temperature guard column from injector to analytical column was used. All

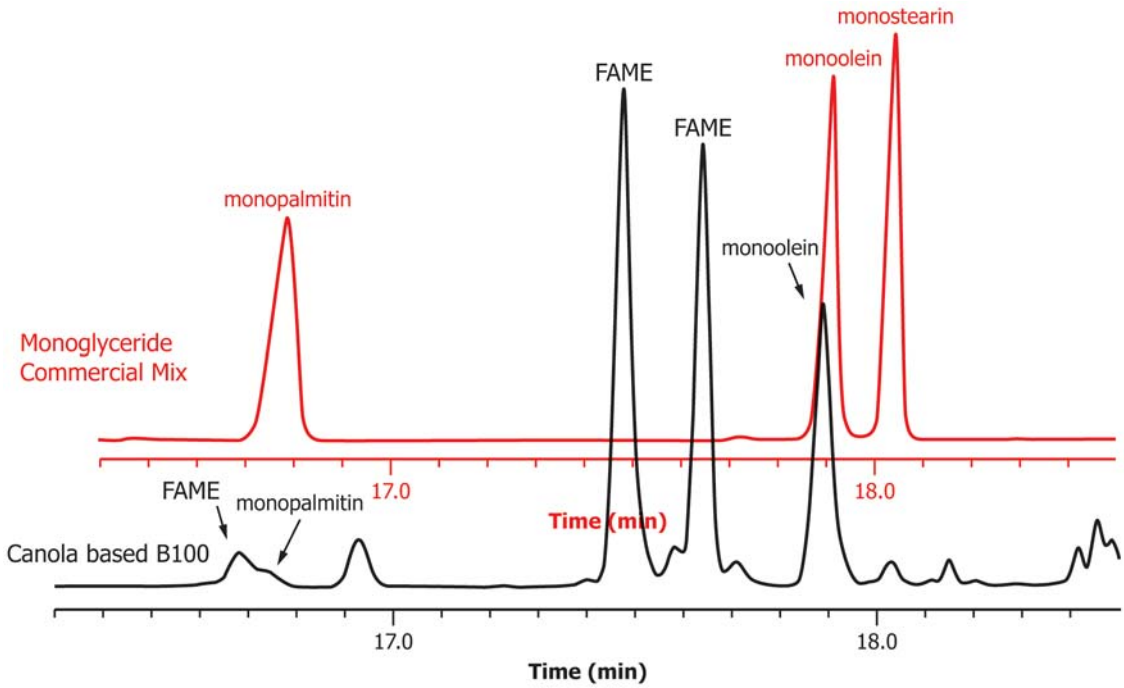

FIG. X1.1 Overlay of Canola based B100 and Monoglyceride Standard Solution

chromatograms, except where noted, were collected using helium carrier gas setting of constant flow of 3 mL/min. This is done to aid in consistency of the example chromatograms. Chromatograms may differ in different laboratories from those shown, depending on the chromatographic system and configuration used.

X1.2 Reagents and Materials

X1.2.1 *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.

X1.2.2 *1-Monooleoyl-rac-glycerol (monoolein),* CAS 111-03-5.

X1.2.3 *1-Monopalmitoyl-rac-glycerol (monopalmitin),* reagent grade, CAS 542-44-9.

X1.2.4 *1-Monostearoyl-rac-glycerol (monostearin),* CAS 123-94-4.

X1.2.5 *Pyridine,* reagent grade.

X1.2.6 *N-Methyl-N-trimethylsilultrifluoroacetamide (MSTFA),* reagent grade.

X1.2.7 *n-Heptane,* reagent grade.

X1.3 Preparation of Monoglyceride Standard

X1.3.1 The use of a reference standard of monoglycerides is strongly suggested for identification of the individual monoglycerides in B100. This reference standard can be used to determine the exact retention times of the monoglyceride species, which can then be applied to the B100 chromatogram to assist in peak identification. Figs. X1.1 and X1.2 show an overlay of a commercially prepared monoglyceride standard and two typical B100 chromatograms.

X1.3.1.1 *Monoglyceride Mixture*—A standard monoglyceride mix, containing monoolein, monostearin, and monopalmitin should be prepared. The mixture may be purchased commercially or prepared prior to analysis. In either case, the monoglycerides should be roughly 100 mg each dissolved in 10 mL pyridine. This mixture will be used to make the standard.

X1.3.2 *Preparation of Monoglyceride Standard*—Transfer 100 µL of monoglyceride mixture (X1.3.1.1) and 100 µL MSTFA into a 10 mL screw cap vial. Avoid contact with moisture. Close vial with PTFE-lined lid and shake. Allow the vial to stand for 15 min to 20 min at room temperature. Add approximately 8 mL n-heptane to the vial and shake.

X1.3.3 *Chromatographic Analysis*—If using an automatic sampler, transfer an aliquot of the solution into a glass GC vial and seal with a PTFE-fluorocarbon lined cap. Analyze the monoglyceride standard following Section 10, Procedure.

X1.4 Variations in Chromatography Due to Constant Pressure Operation Compared to Constant Flow Operation

X1.4.1 Options in many gas chromatographs may allow for the carrier gas flow to be defined by constant pressure or constant flow. Electronic pressure control, found on many newer systems, may allow for constant flow or constant pressure operation. In general, older systems, unless modified to include electronic pressure control, may only allow for constant pressure operation.

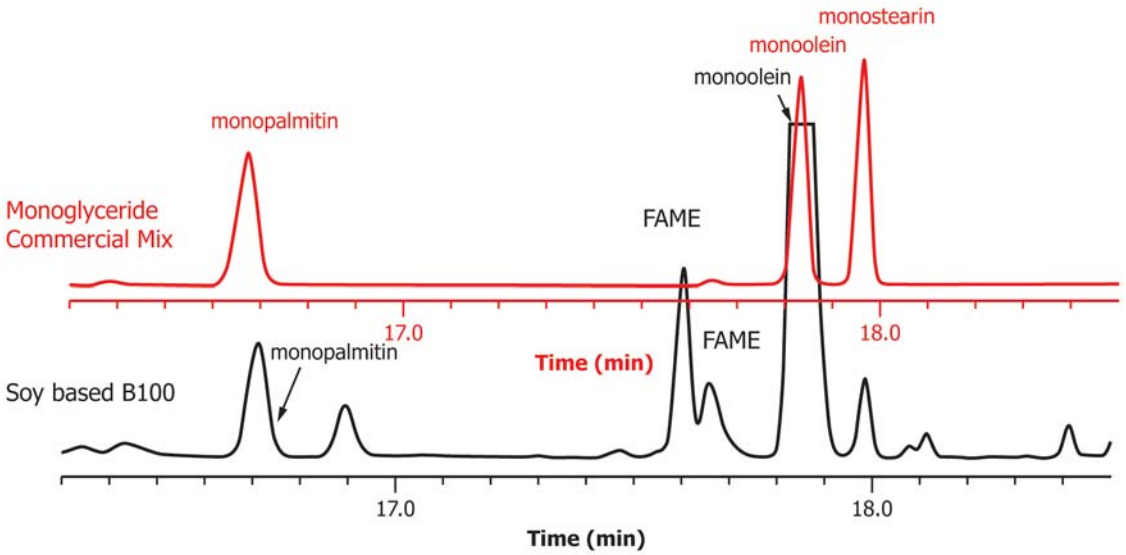

FIG. X1.2 Overlay of Soy based B100 and Monoglyceride Standard Solution

X1.4.2 Depending on the operation of the chromatographic system, the regions of interest (monoglyceride, diglyceride, and triglyceride) may not match those outlined in the method. Fig. X1.3 illustrates a sample injected, first using constant flow control, then using constant pressure control; all other conditions were identical between runs. Note that the retention times of the peaks shift depending on the operation.

X1.5 Additional Example Chromatograms

X1.5.1 All samples were run using helium carrier gas with constant flow through the column of 3 mL/min. Figs. X1.4-X1.6 provide example chromatograms of various B100 feedstocks to show some of the variability that may be experienced when running this method. Peak identifications are also shown.

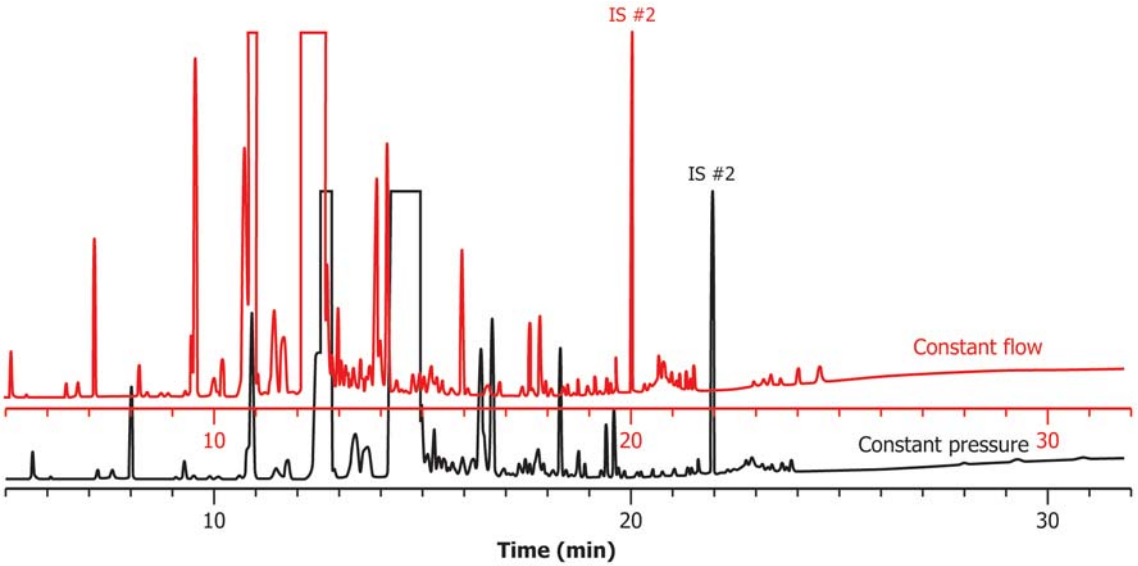

FIG. X1.3 Overlay of B100 sample derived from mixed feedstocks. The top chromatogram was collected with constant flow setting at 3 mL/min and bottom chromatogram was collected with constant pressure, measured as 3 mL/min at 50 °C. All other parameters remained the same

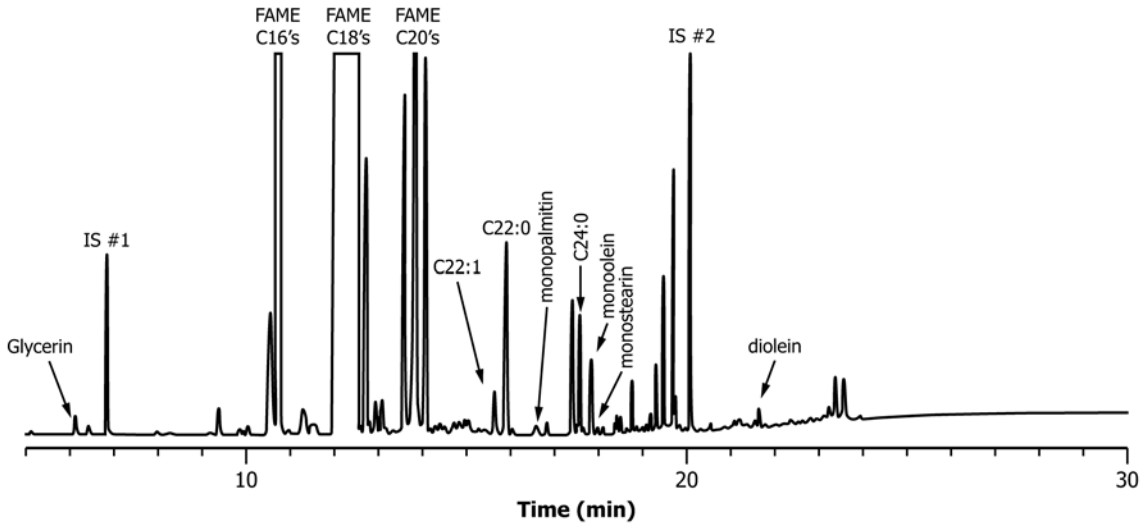

FIG. X1.4 Canola based B100 example chromatogram

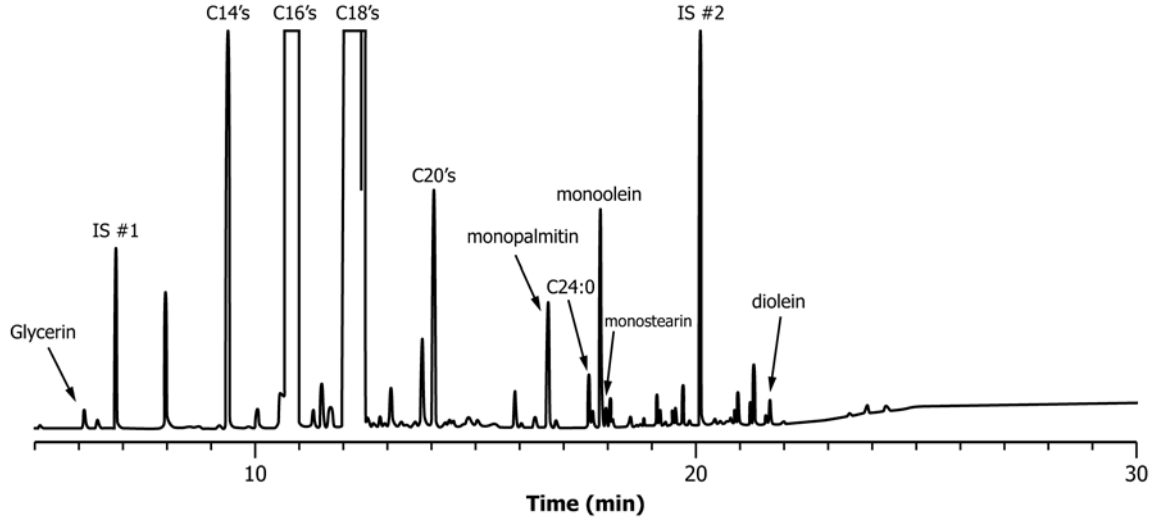

FIG. X1.5 Palm based B100 example chromatogram

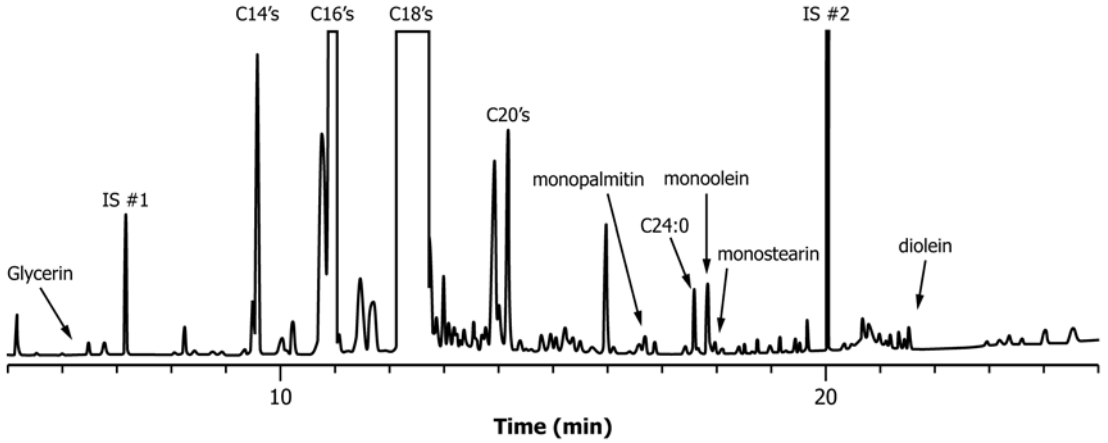

FIG. X1.6 Mixed feedstock based B100 example chromatogram

SUMMARY OF CHANGES

Subcommittee D02.04 has identified the location of selected changes to this standard since the last issue (D6584–13$^{\varepsilon1}$) that may impact the use of this standard. (Approved June 1, 2017.)

(1) Revised Eq 9 in subsection 11.1.3.