Designation: D7371 – 07

Standard Test Method for Determination of Biodiesel (Fatty Acid Methyl Esters) Content in Diesel Fuel Oil Using Mid Infrared Spectroscopy (FTIR-ATR-PLS Method)[1]

This standard is issued under the fixed designation D7371; 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 determination of the content of fatty acid methyl esters (FAME) biodiesel in diesel fuel oils. It is applicable to concentrations from 1.00 to 20 volume % (see Note 1). This procedure is applicable only to FAME. Biodiesel in the form of fatty acid ethyl esters (FAEE) will cause a negative bias.

NOTE 1—Using the proper ATR sample accessory, the range maybe expanded from 1 to 100 volume %, however precision data is not available above 20 volume %.

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

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]

D975 Specification for Diesel Fuel Oils

D976 Test Method for Calculated Cetane Index of Distillate Fuels

D1298 Test Method for Density, Relative Density (Specific Gravity), or API Gravity of Crude Petroleum and Liquid Petroleum Products by Hydrometer Method

D4052 Test Method for Density, Relative Density, and API Gravity of Liquids by Digital Density Meter

D4057 Practice for Manual Sampling of Petroleum and Petroleum Products

D4177 Practice for Automatic Sampling of Petroleum and Petroleum Products

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

D4737 Test Method for Calculated Cetane Index by Four Variable Equation

D5854 Practice for Mixing and Handling of Liquid Samples of Petroleum and Petroleum Products

D6299 Practice for Applying Statistical Quality Assurance and Control Charting Techniques to Evaluate Analytical Measurement System Performance

D6751 Specification for Biodiesel Fuel Blend Stock (B100) for Middle Distillate Fuels

E168 Practices for General Techniques of Infrared Quantitative Analysis

E1655 Practices for Infrared Multivariate Quantitative Analysis

E2056 Practice for Qualifying Spectrometers and Spectrophotometers for Use in Multivariate Analyses, Calibrated Using Surrogate Mixtures

3. Terminology

3.1 *Definitions:*

3.1.1 *biodiesel*, *n*—a fuel comprised of mono-alkyl esters of long chain fatty acids derived from vegetable oils or animal fats, designated B100. **D6751**

3.1.2 *biodiesel blend, BXX*, *n*—a blend of biodiesel fuel with petroleum-based diesel fuel.

3.1.2.1 *Discussion*—In the abbreviation BXX, the XX represents the volume percentage of biodiesel fuel in the blend. **D6751**

3.1.3 *diesel fuel*, *n*—petroleum-based middle distillate fuel.

3.1.4 *multivariate calibration*, *n*—process for creating a model that relates component concentrations or properties to the absorbances of a set of known reference samples at more than one wavelength or frequency. **E1655**

3.1.4.1 *Discussion*—The resultant multivariate calibration model is applied to the analysis of spectra of unknown samples to provide an estimate of the component concentration or property values for the unknown sample.

3.1.4.2 *Discussion*—The multivariate calibration algorithm employed in this test method is partial least square (PLS) as defined in Practices E1655.

3.2 *Abbreviations:*

[1] This test method is under the jurisdiction of ASTM Committee D02 on Petroleum Products and Lubricants and is the direct responsibility of Subcommittee D02.04.0F on Absorption Spectroscopic 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.

ATR = attenuated total reflectance
Bxx = see 3.1.2
FAEE = fatty acid ethyl esters
FAME = fatty acid methyl esters
FTIR = Fourier transform infrared
mid-IR = mid infrared
PLS = partial least square
ULSD = ultra low sulfur diesel

4. Summary of Test Method

4.1 A sample of diesel fuel, biodiesel, or biodiesel blend is introduced into a liquid attenuated total reflectance (ATR) sample cell. A beam of infrared light is imaged through the sample onto a detector, and the detector response is determined. Wavelengths of the absorption spectrum that correlate highly with biodiesel or interferences are selected for analysis. A multivariate mathematical analysis converts the detector response for the selected areas of the spectrum from an unknown to a concentration of biodiesel.

4.2 This test method uses Fourier transform mid-IR spectrometer with an ATR sample cell. The absorption spectrum shall be used to calculate a partial least square (PLS) calibration algorithm.

5. Significance and Use

5.1 Biodiesel is a fuel commodity primarily used as a value-added blending component with diesel fuel.

5.2 This test method is applicable for quality control in the production and distribution of diesel fuel and biodiesel blends containing FAME.

6. Interferences

6.1 The hydrocarbon composition of diesel fuel has a significant impact on the calibration model. Therefore, for a robust calibration model, it is important that the diesel fuel in the biodiesel fuel blend is represented in the calibration set.

6.2 Proper choice of the apparatus, design of a calibration matrix, utilization of multivariate calibration techniques, and evaluation routines as described in this standard can minimize interferences.

6.3 *Water Vapor Interference*—The calibration and analysis bands in A1.2 lie in regions where significant signals due to water vapor can appear in the infrared spectrum. This shall be accounted for to permit calibration at the low end concentrations.

NOTE 2—Ideally, the spectrometer should be purged with dry air or nitrogen to remove water vapor. The purge should be allowed to stabilize over several hours before analytical work is pursued, due to the rapid changes in the air moisture content within the spectrometer during early stages of the purge. In cases where water vapor prevention or elimination is not possible using a purge, the operator should measure a reference background spectrum for correction of the ratioed spectrum for each sample spectrum measured. This operation is generally automated in today's spectrometer systems and the operator should consult the manufacturer of the spectrometer for specific instructions for implementing automated background correction routines. The spectrometer should be sealed and desiccated to minimize the affect of water vapor variations, and any accessory should be sealed to the spectrometer.

TABLE 1 Attenuated Total Reflectance (ATR) Conical Cells Specification

ATR element material	ZnSe
beam condensing optics	conical, non-focusing optics integral to cell body
element configuration	circular cross section with coaxial conical ends
cone half angle	60°
element length	36.83 to 39.37 mm (1.45 to 1.55 in.)
element diameter	3.175 mm (0.125 in.)
angle of incidence at sample interface	53.8°
maximum range of incidence angles	± 1.5°
standard absorbance (1428 cm^{-1} band of acetone)	0.38 ± 0.02 AU
material of construction	316 stainless steel
seals	Chemrez or Kalrez o-rings[A]

[A] Trademarks of Chemrez, Inc. and Dupont Performance Elastomers L.L.C.

6.4 *Fatty Acid Ethyl Esters (FAEE) Interference*—The presence of FAEE in the composition of the biodiesel will result in an overall lower concentration measurement of biodiesel content. Outlier statistical results may be a useful tool for determining high concentration FAEE content (for additional FAEE information, see research report referenced in Section 15).

6.5 *Undissolved Water*—Samples containing undissolved water will result in erroneous results. Filter cloudy or water saturated samples through a dry filter paper until clear prior to their introduction into the instrument sample cell.

7. Apparatus

7.1 *Mid-IR Spectrometric Analyzer*:

7.1.1 *Fourier Transform Mid-IR Spectrometer*—The type of apparatus suitable for use in this test method employs an IR source, a liquid attenuated total internal reflection cell, a scanning interferometer, a detector, an A-D converter, a microprocessor, and a method to introduce the sample. The following performance specifications shall be met:

Scan Range	4000 to 650 cm^{-1}
Resolution	4 cm^{-1}

7.1.2 The noise level shall be established by acquiring a single beam spectrum using air or nitrogen. The single beam spectrum obtained can be the average of multiple of FTIR scans but the total collection time shall not exceed 60 seconds. If interference from water vapor or carbon dioxide is a problem, the instrument shall be purged with dry air or nitrogen. The noise of the spectrum at 100 % transmission shall be less than 0.3 % in the region from 1765 to 1725 cm^{-1}.

7.2 *Absorption Cell*, multi-bounce (multi-reflections) attenuated total reflectance cell. It shall meet one of the following requirements:

7.2.1 *Conical Attenuated Total Reflectance (ATR) Cell*, having similar specifications defined in Table 1. This cell is suitable for the low, medium, and high concentration ranges.

7.2.2 *Horizontal Attenuated Total Reflectance (ATR) Cell*, with ZnSe element ATR mounted on a horizontal plate. The absorbance at 1745 cm^{-1} shall not exceed 1.2 absorbance units for the highest concentration calibration standard used in the calibration range. Therefore, for higher concentration measurements, careful consideration of element length and face angle shall be made to maximize sensitivity without exceeding 1.2 absorbance units at 1745 cm^{-1}.

8. Reagents and Materials

8.1 *Purity of Reagents*—Spectroscopic grade (preferred) or reagent grade chemicals shall be used in tests. Unless otherwise indicated, it is intended that all reagents shall 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 sufficiently high purity to permit its use without lessening the accuracy of the determination.

8.1.1 *B100 (Neat Biodiesel)*—Used for calibration, qualification, and quality control standards shall be compliant with Specification D6751. The B100 shall be fatty acid methyl esters. Soy methyl ester (SME) was used in calibration standards for developing the precision of this test method. Esters derived from other feedstocks, for example animal fats, canola oil, jatropha oil, palm oil, rapeseed oil, and yellow grease may be used. Standards made with yellow grease methyl esters should not represent more than 50 % of the number of the calibration standards. A BQ-9000 certified producer for the biodiesel is recommended to ensure quality of product. See Annex A2 for further discussion.

8.1.2 *Middle Distillate Fuel*—Used for calibration, qualification, and quality control standards shall be compliant with Specification D975, free of biodiesel or biodiesel oil precursor, or both. As far as possible, middle distillate fuel shall be representative of petroleum base stocks anticipated for blends to be analyzed (crude source, 1D, 2D, blends, winter/summer cuts, low aromatic content, high aromatic content, and the like). See Annex A2 for calibration set.

8.1.3 *Diesel Cetane Check Fuel*—Low (DCCF-Low).[4] (See A2.2 for alternative material.)

8.1.4 *Diesel Cetane Check Fuel*—High (DCCF-High).

8.1.5 *Diesel Cetane Check Fuel*—Ultra High (DCCF-Ultra High).

8.1.6 *Acetone [67-64-1]*—Reagent grade.

8.1.7 *Toluene [108-88-3]*—Reagent grade.

8.1.8 *Methanol [67-56-1]*—Reagent grade.

8.1.9 *Triple Solvent*—A mixture of equal parts by volume of toluene, acetone, and methanol.

9. Sampling and Sample Handling

9.1 *General Requirements*:

9.1.1 Fuel samples to be analyzed by this test method shall be sampled using procedures outlined in Practice D4057 or Practice D4177, where appropriate. Do not use "sampling by water displacement." FAME is more water-soluble than the hydrocarbon base in a biodiesel blend.

9.1.2 Protect samples from excessive temperatures prior to testing.

9.1.3 Do not test samples stored in leaky containers. Discard and obtain a new sample if leaks are detected.

9.2 *Sample Handling During Analysis*:

9.2.1 When analyzing samples using the FTIR, the sample temperature needs to be within the range of 15 to 27°C. Equilibrate all samples to the temperature of the laboratory (15 to 27°C) prior to analysis by this test method.

9.2.2 After analysis, if the sample is to be retained, reseal the container before storing.

10. Calibration and Qualification of the Apparatus

10.1 Before use, the instrument needs to be calibrated according to the procedure described in Annex A1. This calibration can be performed by the instrument manufacturer prior to delivery of the instrument to the end user. If, after maintenance, the instrument calibration is repeated, the qualification procedure is also repeated.

10.2 Before use, the instrument is qualified according to the procedure described in Annex A1. The qualification need only be carried out when the instrument is initially put into operation, recalibrated, or repaired.

11. Quality Control Checks

11.1 Confirm the in-statistical-control status of the test method each day it is used by measuring the biodiesel concentration of at least one quality control sample that is similar in composition and matrix to samples routinely analyzed. For details on quality control sample selection, preparation, testing, and control charting, refer to Practice D6299.

11.2 A system that is found to be out of statistical control cannot be used until the root cause(s) of out-of-control is identified and corrected.

11.3 If correction of out-of-control behavior requires repair to the instrument or recalibration of the instrument, the qualification of instrument performance described in A1.3 shall be performed before the system is used to measure the biodiesel content of samples.

12. Procedure

12.1 Equilibrate the samples to between 15 and 27°C before analysis.

12.2 Clean the sample cell of any residual fuel according to the manufacturer's instructions. Remove the fuel by flushing the cell with sufficient solvent or the subsequent sample to ensure complete washing. For difficult to remove substances like B100, precede flushing with triple solvent. Evaporate the residual solvent with either dry air or nitrogen.

12.3 Obtain a baseline spectrum in the manner established by the manufacturer of the equipment.

12.4 Prior to the analysis of unknown test samples, establish that the equipment is running properly by collecting the spectrum of the quality control standard(s) and comparing the estimated biodiesel concentration(s) to the known value(s) for the QC standard(s). Introduce enough standard into the cell to ensure that the cell is washed by at least three times the cell volume.

[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.

[4] The sole source of supply of the material known to the committee at this time is Chevron Phillips Chemical Company LLC, 10001 Six Pines Drive, The Woodlands, TX 77380. If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.

12.5 Introduce the unknown fuel sample in the manner established by the manufacturer. Introduce enough of the fuel sample to the cell to ensure that the cell is washed by at least three times the cell volume.

NOTE 3—Biodiesel and biodiesel blends containing high concentrations of biodiesel are difficult to remove from the cell surface of an ATR crystal. Flush several times with sample or use a solvent rinse between samples. When in doubt, repeat steps 12.5 through 12.7 and compare the results to ensure adequate rinsing occurred.

12.6 Obtain the spectral response of the fuel sample.

12.6.1 Acquire the digitized spectral data for the fuel sample over the frequency region from 4000 to 650 cm^{-1}.

12.7 Determine the biodiesel concentration (volume %) according to the appropriate calibration equation developed in Annex A1.

12.7.1 Determine the biodiesel concentration using the calibration models developed in A1.2.4 by following the steps outlined as follows:

12.7.1.1 Estimate the biodiesel concentration in the fuel sample by applying the low calibration (see A1.2.4.1) to the spectrum in the region of 1800 to 1692 cm^{-1} and 1327 to 940 cm^{-1} using no baseline correction.

12.7.1.2 If the estimated biodiesel concentration determined in 12.7.1.1 is equal to or less than 10.00 volume %, determine the biodiesel concentration by applying the low calibration (see A1.2.4.1).

12.7.1.3 If the estimated biodiesel concentration determined in 12.7.1.2 is greater than 10.00 volume %, estimate the biodiesel concentration by applying the medium calibration (see A1.2.4.2) to the spectrum in the region of 1800 to 1700 cm^{-1} and 1399 to 931 cm^{-1} using no baseline correction.

12.7.1.4 If the value estimated by application of the medium calibration determined in 12.7.1.3 is less than or equal to 10.50 volume %, report the value determined by the low calibration (even if the value is greater than 10.5 volume %). For estimated values greater than 10.50 volume % and less than or equal to 30.00 volume % determined in 12.7.1.3, report the value obtained.

12.7.1.5 If the estimated biodiesel concentration determined in 12.7.1.4 is greater than 31.00 volume %, estimate the biodiesel concentration by applying the high calibration (see A1.2.4.3) to the spectrum in the region of 1851 to 1670 cm^{-1} and 1371 to 1060 cm^{-1} using no baseline correction.

12.7.1.6 If the value estimated by application of the high calibration determined in 12.7.1.5 is less than or equal to 31.00 volume %, report the value determined by the medium calibration (even if the value is greater than 31.00 volume %). For estimated values greater than 31.00 volume % (determined in 12.7.1.5), report the value obtained.

NOTE 4—Clean cell thoroughly after use. Occasionally, clean cell of any water soluble substances by first cleaning with acetone. Place a solution of 30 % alcohol (ethyl or methyl) in water in the cell and let it soak for at least one hour. Finally, clean cell with acetone and dry. No acids or bases should be used in cleaning ZnSe elements.

13. Calculation

13.1 *Conversion to Volume % of Biodiesel*—To convert the calibration and qualification standards to volume % use Eq 1.

TABLE 2 Repeatability as a Function of Concentration

Biodiesel Concentration (volume %)	Repeatability (volume %)
1.00	0.24
2.00	0.25
5.00	0.30
10.00	0.37
20.00	0.53
50.00	not determined
90.00	not determined
100.00	not determined

$$V_b = M_b(D_f/D_b) \quad (1)$$

where:

V_b = biodiesel volume %,
M_b = biodiesel mass %,
D_f = relative density at 15.56°C of the calibration or qualification standard being tested as determined by Practice D1298 or Test Method D4052, and
D_b = B100 biodiesel blend stock relative density at 15.56°C of the calibration or qualification standard being tested as determined by Practice D1298 or Test Method D4052.

14. Report

14.1 Report the following information:

14.1.1 Volume % biodiesel by Test Method D7371, to the nearest 0.01 %.

15. Precision and Bias [5]

15.1 Interlaboratory tests were carried out in 5 laboratories using 16 samples that covered the range from 1 to 20 volume %. The precision of the test method as obtained by statistical examination of interlaboratory results is summarized in Tables 2 and 3.

15.2 *Repeatability*—For biodiesel concentrations between 1.00 and 20.00 volume %, the difference between successive test results obtained by the same operator with the same apparatus under constant operating conditions on identical test samples would, in the long run, and in the normal and correct operation of the test method, exceed the following values only in one case in twenty:

$$\text{Repeatability} = 0.01505\ (X + 14.905)\ \text{volume}\ \% \quad (2)$$

where:

X = biodiesel concentration determined.

15.3 *Reproducibility*—For biodiesel concentrations between 1.00 and 20.00 volume %, the difference between two single and independent results obtained by different operators working in different laboratories on identical test samples would, in the long run, and in the normal and correct operation of the test method, exceed the following values only in one case in twenty:

$$\text{Reproducibility} = 0.04770\ (X + 14.905)\ \text{volume}\ \% \quad (3)$$

[5] Supporting data have been filed at ASTM International Headquarters and may be obtained by requesting Research Report RR:D02-1624.

TABLE 3 Reproducibility as a Function of Concentration

Biodiesel Concentration (volume %)	Reproducibility (volume %)
1.00	0.76
2.00	0.81
5.00	0.95
10.00	1.19
20.00	1.66
50.00	not determined
90.00	not determined
100.00	not determined

where:

X = biodiesel concentration determined.

15.4 *Bias*—No information can be presented on the bias of the procedure employed in this test method because no primary reference material having an accepted reference value is currently available.

16. Keywords

16.1 biodiesel; biodiesel blend; biodiesel (FAME) content; FAME; fatty acid methyl esters; infrared spectroscopy

ANNEXES

(Mandatory Information)

A1. CALIBRATION AND QUALIFICATION OF THE APPARATUS

A1.1 *Calibration Matrix*—Calibration and validation standards shall be prepared in accordance with Practice D4307 or appropriately scaled for larger blends and Practice D5854, where appropriate. Use blend components that are known to be fully compliant with Specification D975 (for base petroleum diesel components) and Specification D6751 (for B100 biodiesel components). See Annex A2 for selecting blend components.

A1.1.1 *Calibration Matrices*—To obtain best precision and accuracy of calibration using the PLS model, prepare three biodiesel calibration sets as set forth in Table A1.1. The first set (Set A) contains samples with biodiesel concentrations between 0.00 and 10.00 volume %. The second set (Set B) contains samples with biodiesel concentrations from 10.00 to 30.00 volume %. An optional third set (Set C) contains samples with biodiesel concentrations from 30.00 to 100 volume %.

A1.1.2 Measure the density of each of the components to be mixed and of the calibration standards according to either Test Method D1298 or Test Method D4052.

A1.1.3 For each of the calibration standards, convert the mass % biodiesel to volume % biodiesel using Eq 1.

A1.2 *Calibration*:

A1.2.1 The instrument is calibrated in accordance with the mathematics as outlined in Practices E1655. This practice serves as a guide for the multivariate calibration of infrared spectrometers used in determining the physical characteristics of petroleum and petrochemical products. The procedures describe treatment of the data, development of the calibration, and qualification of the instrument.

A1.2.2 Equilibrate all samples to the temperature of the laboratory (15 to 27°C) prior to analysis. Fill the sample cell with the calibration standards in accordance with Practices E168 or in accordance with the manufacturer's instructions.

A1.2.3 For each of the calibration standards, acquire the digitized spectral data over the frequency region from 4000 to 650 cm^{-1}. The infrared spectrum is the negative logarithm of the ratio of the single beam infrared spectrum obtained with a sample and the single beam FTIR spectrum with dry air (or nitrogen).

A1.2.4 Two separate partial least square (PLS) calibrations will be developed and an optional third calibration.

A1.2.4.1 Develop the first calibration, referred to as the low calibration (0 to 10.00 volume %), using spectra obtained from the samples in calibration set a detailed in Table A1.1. This calibration relates the spectrum to the biodiesel concentration (volume %). Use data in the region of 1800 to 1692 and 1327 to 940 cm^{-1} to develop the low calibration range using no baseline correction. Use mean centering and three latent variables (factors) in developing the model.

NOTE A1.1—If, for a particular instrument or instrument type, analysis of an independent validation set via the methodology described in Practices E1655 demonstrates that models based on 4 latent variables provides a meaningfully lower standard error of validation than models based on 3 latent variables, then models based on 4 factors may be used. The reason for using 4 latent variables shall be documented.

A1.2.4.2 Develop the second calibration, referred to as the medium calibration (10.00 to 30.00 volume %), using spectra obtained from all of the samples in calibration Set B as detailed in Table A1.1. This calibration relates the spectrum to the biodiesel concentration (volume %). Use data in the region of 1800 to 1700 cm^{-1} and 1399 to 931 cm^{-1} to develop the medium calibration range using no baseline correction. Use mean centering and three latent variables (factors) in developing the model.

NOTE A1.2—If, for a particular instrument or instrument type, analysis of an independent validation set via the methodology described in Practices E1655 demonstrates that models based on 4 latent variables provides a meaningfully lower standard error of validation than models based on 3 latent variables, then models based on 4 factors may be used. The reason for using 4 latent variables shall be documented.

A1.2.4.3 Develop the third calibration, (optional; using samples over the range 30.00 to 100.0 volume %), referred to as the high calibration, using spectra obtained from all of the samples in calibration Set C as detailed in Table A1.1. This calibration relates the spectrum to the biodiesel concentration

TABLE A1.1 Instrument Calibration Sets A, B, and C

Sample	Biodiesel [vol %]	Solvent	Set A	Set B	Set C
1	0.00	DCCF-Low	X		
2	0.25	DCCF-Low	X		
3	0.50	DCCF-Low	X		
4	1.00	DCCF-Low	X		
5	2.50	DCCF-Low	X		
6	5.00	DCCF-Low	X		
7	7.50	DCCF-Low	X		
8	10.00	DCCF-Low	X	X	
9	12.50	DCCF-Low		X	
10	15.00	DCCF-Low		X	
11	17.50	DCCF-Low		X	
12	20.00	DCCF-Low		X	
13	25.00	DCCF-Low		X	
14	30.00	DCCF-Low		X	X
15	50.00	DCCF-Low			X
16	70.00	DCCF-Low			X
17	80.00	DCCF-Low			X
18	90.00	DCCF-Low			X
19	95.00	DCCF-Low			X
20	97.00	DCCF-Low			X
21	99.00	DCCF-Low			X
22	99.80	DCCF-Low			X
23	0.00	DCCF-High	X		
24	0.25	DCCF-High	X		
25	0.50	DCCF-High	X		
26	1.00	DCCF-High	X		
27	2.50	DCCF-High	X		
28	5.00	DCCF-High	X		
29	7.50	DCCF-High	X		
30	10.00	DCCF-High	X	X	
31	12.50	DCCF-High		X	
32	15.00	DCCF-High		X	
33	17.50	DCCF-High		X	
34	20.00	DCCF-High		X	
35	25.00	DCCF-High		X	
36	30.00	DCCF-High		X	X
37	50.00	DCCF-High			X
38	70.00	DCCF-High			X
39	80.00	DCCF-High			X
40	90.00	DCCF-High			X
41	95.00	DCCF-High			X
42	97.00	DCCF-High			X
43	99.00	DCCF-High			X
44	99.80	DCCF-High			X
45	0.00	DCCF-Ultra High	X		
46	0.25	DCCF-Ultra High	X		
47	0.50	DCCF-Ultra High	X		
48	1.00	DCCF-Ultra High	X		
49	2.50	DCCF-Ultra High	X		
50	5.00	DCCF-Ultra High	X		
51	7.50	DCCF-Ultra High	X		
52	10.00	DCCF-Ultra High	X	X	
53	12.50	DCCF-Ultra High		X	
54	15.00	DCCF-Ultra High		X	
55	17.50	DCCF-Ultra High		X	
56	20.00	DCCF-Ultra High		X	
57	25.00	DCCF-Ultra High		X	
58	30.00	DCCF-Ultra High		X	X
59	50.00	DCCF-Ultra High			X
60	70.00	DCCF-Ultra High			X
61	80.00	DCCF-Ultra High			X
62	90.00	DCCF-Ultra High			X
63	95.00	DCCF-Ultra High			X
64	97.00	DCCF-Ultra High			X
65	99.00	DCCF-Ultra High			X
66	99.80	DCCF-Ultra High			X
67	Mfg'r 1 100.00	—			X
68	Mfg'r 2 100.00	—			X
69	Mfg'r 3 100.00	—			X
70	Mfg'r 4 100.00	—			X

TABLE A1.2 Pooled Standard Errors of Qualification

	Calibration
PSEQ	0.21
DOF(PSEQ)	56

TABLE A1.3 Critical *F* Value

Denominator DOF(PSEQ)	Critical *F* Value
20	1.76
21	1.75
22	1.74
23	1.72
24	1.71
25	1.70
30	1.66
35	1.63
40	1.61

(volume %). Use data in the region of 1851 to 1670 and 1371 to 1060 cm^{-1} to develop the high calibration range using no baseline correction. Use mean centering and three latent variables (factors) in developing the model. This calibration model is optional and its use can be limited by the cell accessory used.

NOTE A1.3—If, for a particular instrument or instrument type, analysis of an independent validation set via the methodology described in Practices E1655 demonstrates that models based on 4 latent variables provides a meaningfully lower standard error of validation than models based on 3 latent variables, then models based on 4 factors may be used. The reason for using 4 latent variables shall be documented.

A1.3 *Qualification of Instrument Performance*—Once calibration has been established, the individual calibrated instrument is qualified to ensure that the instrument accurately and precisely measures biodiesel in the presence of typical compression-ignition engine fuel compounds that, in typical concentrations, present spectral interferences. This qualification need only be carried out when the instrument is initially put into operation, recalibrated, or repaired.

A1.3.1 *Preparation of Qualification Samples*—Prepare multicomponent qualification standards of the biodiesel by mass according to Practice D4307 (or appropriately scaled for larger blends), or Practice D5854, where appropriate. These standards shall be similar to, but not the same as, the mixtures established for the calibration set used in developing the calibration. Prepare the qualification samples so as to vary the concentrations of biodiesel and of the interfering components over a range that spans at least 95 % of that for the calibration standards. The numbers of required standards are suggested by Practice E2056 and, in general, will be three times the number of independent variables in the calibration equation. For a three component PLS model, a minimum of 20 qualification standards are required.

A1.3.2 *Acquisition of Qualification Data*—For each of the qualification standards, measure the biodiesel concentration, expressed in volume %, according to the procedure established in Section 12. The adequacy of the instrument performance is determined following the procedures similar to those described in Practice E2056.