Designation: D8144 – 22

Standard Test Method for Separation and Determination of Aromatics, Nonaromatics, and FAME Fractions in Middle Distillates by Solid-Phase Extraction and Gas Chromatography[1]

This standard is issued under the fixed designation D8144; 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 separation and determination of representative aromatics, nonaromatics, and fatty acid methyl ester (FAME) fractions in middle distillates that boil between 170 °C and 400 °C, including biodiesel blends with up to 20 % by volume of FAME, by solid phase extraction and gas chromatography.

1.2 This test method provides two procedures, A and B. Procedure A is applicable to the petroleum-based middle distillates fuel, and Procedure B is applicable to the biodiesel blends with up to 20 % by volume of FAME.

1.3 This test method is applicable to middle distillates samples with aromatics content ranging from 5 % to 50 % by mass and biodiesel blends with FAME content in the range of 0.5 % to 20 % by volume. This test method may apply to concentrations outside these ranges, but the precision has not been determined.

1.4 For Procedure B, biodiesels in the form of fatty acid ethyl ester (FAEE) can also fully elute into the FAME fraction, and they have the similar FID (flame ionization detector) relative response factors with that of FAME. The determined content of FAME fractions are the sum of concentrations of FAME and FAEE by this test method (see 3.2.5).

1.5 From the investigation results obtained for FAME determination, the low concentrations of monoglycerides (usually less than 0.5 % by mass in biodiesel blends) are not detectable under the gas chromatographic (GC) condition of this test method and will not interfere with the determination of FAME by Procedure B. As a result, biodiesel blends, conforming to the requirements of Specification D7467, containing up to 20 % by volume of biodiesel blendstock meeting the requirements in Specification D6751, typically contain concentrations of monoglycerides of less than 0.1 % by mass. The diglycerides and triglycerides, if present, are not detected under the GC condition of this test method due to their higher boiling points.

NOTE 1—If a sample is suspected of containing an abnormal FAME biodiesel feedstock than specified in Specification D6751, for example, a sample contaminated with vegetable oil with a high level of total triglycerides, the content of mono-, di-, or tri-glycerides in the isolated FAME fraction may be determined using Test Method D6584. Samples containing biodiesels with a high amount of glycerides than specified in Specification D6751 may contaminate the GC column and not recommended for this test method.

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

1.7 *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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.8 *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]

D2425 Test Method for Hydrocarbon Types in Middle Distillates by Mass Spectrometry

D2549 Test Method for Separation of Representative Aromatics and Nonaromatics Fractions of High-Boiling Oils by Elution Chromatography

D2887 Test Method for Boiling Range Distribution of Petroleum Fractions by Gas Chromatography

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

[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 Subcommittee 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

D4057 Practice for Manual Sampling of Petroleum and Petroleum Products
D4175 Terminology Relating to Petroleum Products, Liquid Fuels, and Lubricants
D4177 Practice for Automatic Sampling of Petroleum and Petroleum Products
D6299 Practice for Applying Statistical Quality Assurance and Control Charting Techniques to Evaluate Analytical Measurement System Performance
D6584 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
D6751 Specification for Biodiesel Fuel Blend Stock (B100) for Middle Distillate Fuels
D7467 Specification for Diesel Fuel Oil, Biodiesel Blend (B6 to B20)

2.2 *Other Standards:*[3]

EN 14103 Fat and oil derivatives—Fatty Acid Methyl Esters (FAME)—Determination of ester and linolenic acid methyl ester contents
EN 14214 Automotive fuels—Fatty acid methyl esters (FAME) for diesel engines—Requirements and test methods

3. Terminology

3.1 *Definitions:*

3.1.1 For definitions of terms used in this test method, refer to Terminology D4175.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *aromatics fraction, n*—the portion of the sample desorbed with the dichloromethane-ethyl alcohol mixture eluants (Procedure A) and dichloromethane-n-hexane eluants (Procedure B); the aromatics fraction may contain aromatics, condensed naphthenic-aromatics, aromatic olefins, and compounds containing sulfur, nitrogen, and oxygen atoms.

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

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

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

3.2.5 *fatty acid methyl ester fraction, n*—the portion of the diesel fuels blends with fatty acid methyl ester (FAME) eluted with dichloromethane-ethyl alcohol; the FAME fraction may contain FAEE and compounds containing nitrogen and oxygen atoms.

3.2.6 *nonaromatics fraction, n*—the portion of the sample eluted with n-hexane.

3.2.6.1 *Discussion*—The nonaromatics fraction is a mixture of paraffinic and naphthenic hydrocarbons if the sample is a straight-run material. If the sample is a cracked stock, the nonaromatics fraction will also contain aliphatic and cyclic olefins.

3.2.7 *solid phase extraction separating system, n*—a solid-phase extraction cartridge packed with stationary phase material to effectively separate the aromatics, nonaromatics, and other compounds (such as FAME) fractions in middle distillates based on the mechanism of solid phase extraction (SPE).

4. Summary of Test Method

4.1 *Procedure A*—The sample is charged to the top of a SPE column and separated into aromatics and nonaromatics fractions by eluants with different polarities. Two aliquots of internal standards are added to these two fractions and both fractions are analyzed by the gas chromatograph equipped with hydrogen flame ionization detector (GC-FID). The content of the aromatics and nonaromatics are calculated based on the peak areas of the aromatics, nonaromatics, and internal standards.

4.2 *Procedure B*—The sample is charged to the top of a SPE column and separated into aromatics, nonaromatics, and FAME fractions by eluants with different polarities. Three aliquots of internal standards are added to these three fractions. All of these fractions are analyzed by the gas chromatograph equipped with hydrogen flame ionization detector (GC-FID). The content of the aromatics, nonaromatics, and FAME fractions are calculated based on the peak areas of the aromatics, nonaromatics, FAME, and internal standards. The volume percent of FAME is calculated based on the density of sample and mass percent of FAME.

5. Significance and Use

5.1 For the middle distillates whose boiling range is between 170 °C and 400 °C by such distillation methods like Test Method D2887, Procedure A can separate and determine the content of total aromatics and total nonaromatics by SPE and GC analysis of the resulting fractions. The determination of the total content of saturates and aromatics in petroleum middle distillates is useful to investigate the effects of petroleum processes on production of various finished fuels.

5.2 The total aromatics content and polycyclic aromatics content are important to characterize the quality of diesel fuels. This test method is demonstrated to be time-saving and eco-friendly by reducing the amount of reagent consumption and avoiding the necessity of solvent evaporation step as required, for example, in such Test Method D2549.

5.3 The determination of detailed hydrocarbon composition by mass spectrometry requires a preliminary separation of the sample into representative aromatics and nonaromatics, as in Test Method D2425, where Test Method D2549 is used to separate the distillate fuel. The SPE fractionation procedure described herein may provide a suitable fractionation alternative approach for these mass spectrometric types of methods.

5.4 Biodiesel is a blendstock commodity primarily used as a value-added blending component with diesel fuel. Procedure B can provide a separation and determination technique to monitor the FAME content for FAME biodiesel blends.

[3] Available from British Standards Institution (BSI), 389 Chiswick High Rd., London W4 4AL, U.K., http://www.bsigroup.com.

6. Apparatus

6.1 *Solid Phase Extraction Separating System:*

6.1.1 *Solid Phase Extraction (SPE) Column,* [4] as shown in Fig. 1. The SPE column used in Section 11 is 3 mL column packed with 1.5 g stationary phase particles. The stationary phase is a mixture 90:10 by mass of 75 µm to 150 µm silica gel and 75 µm to 150 µm neutral aluminum oxide. Appropriate separation efficiency and activity are required for the SPE column to obtain a satisfactory separation and quantification results. The detailed verification procedure and criteria for SPE column separation efficiency are described in 10.1.

NOTE 2—Any automated solid phase extraction instrument that can perform this separation procedure with the satisfied separation efficiency can also be used.

NOTE 3—The SPE column may be stored in a dry atmosphere as long as its performance meets the specifications; the SPE column is disposable and is used only once.

6.1.2 *Erlenmeyer flask,* 10 mL.

6.1.3 *Syringe,* 2 mL and 0.25 mL.

6.1.4 *Pipette,* 1 mL.

6.1.5 *Analytical Balance,* capable of weighing to the nearest 0.0001 g.

[4] The sole source of supply of the apparatus (Solid Phase Extraction (SPE) Column that meets the requirements for this test method) known to the committee at this time is Research Institute of Petroleum Processing, China Petroleum and Chemical Corporation, 18 Xueyuan Road, Beijing 100083, P. R. China. North American distribution is through Dikma Technologies Inc. 255 Shields Court, Unit A&B, Markham, ON L3R 8V2, Canada, Toll-Free: 1-866-889-9072 or http://www.dikmatech.com. 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.

1 Sample Inlet
2 Adapter
3 Solid Phase Extraction Cartridge
4 Sieve-plate
5 Stationary Phase Particles
6 Sample Outlet

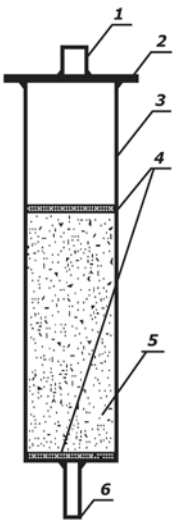

FIG. 1 Solid Phase Extraction Column

6.2 *Gas Chromatographic (GC) System*—The gas chromatographic system shall be equipped with sample inlet system, capillary column, column temperature programmer, FID detector and data acquisition system. Recommended GC operating conditions are given in Table 1. The GC system and operating conditions shall ensure baseline separation of the solvent, sample and internal standard as shown in Fig. 2 and Fig. 3. Any other gas chromatograph instrument and operating conditions capable of yielding equivalent results may be used.

6.2.1 *Sample Introduction System*—Manual or recommended automated liquid syringe injection into a splitting inlet may be employed. The sample amount reaching the column (combination of injection volume and split ratio) should meet the requirement of separation efficiency and linear response calibration range.

6.2.2 *Capillary Column*—This test method is limited to the use of non-polar silica capillary columns. The column and conditions shall provide separation of typical petroleum hydrocarbons in order of increasing boiling point. See Fig. 2 and Fig. 3 for examples of acceptable separation.

6.2.3 *Detector*—This test method requires a flame ionization detector (FID). The detector shall have enough sensitivity, linearity, and stability to meet performance requirements.

6.2.4 *Data Acquisition System*—A computerized data acquisition and reporting system is required to acquire, display, and process GC data. The areas of chromatographic peaks can be determined through either manual or automated peak integration.

TABLE 1 Recommended Operating Conditions of GC[A]

Column	Fused silica WCOT capillary column
Size	30 m × 0.25 mm ID, film thickness 0.25 µm
Stationary phase	Non-polar, such as 100 % dimethyl polysiloxane or 5 % phenyl-methyl polysiloxane
Column Temperature Program	Initial oven temperature 60 °C, initial hold 2 min, program rate 40 °C/min, final oven temperature 300 °C, final hold 5 min
Inlet	
Temperature	300 °C
Split ratio	20:1
Sample size	0.5 µL
Carrier gas	
Type	Helium, Nitrogen, or Hydrogen
Constant Flow Mode	1 mL/min
Detector	
Type	Flame ionization detector (FID)
Temperature	350 °C
Fuel gas	Hydrogen (~30 mL/min)
Oxidizing gas	Air (~300 mL/min)
Make-up gas	~25 mL/min

[A] The operating conditions given in this table are typical and may vary. The length of the WCOT column can be 15 m to 30 m; the inner diameter can be 0.15 mm to 0.32 mm. The suitable oven program can be selected to ensure baseline separation of the solvent, sample ,and internal standard. See Fig. 2 and Fig. 3 for examples of suitable resolution.

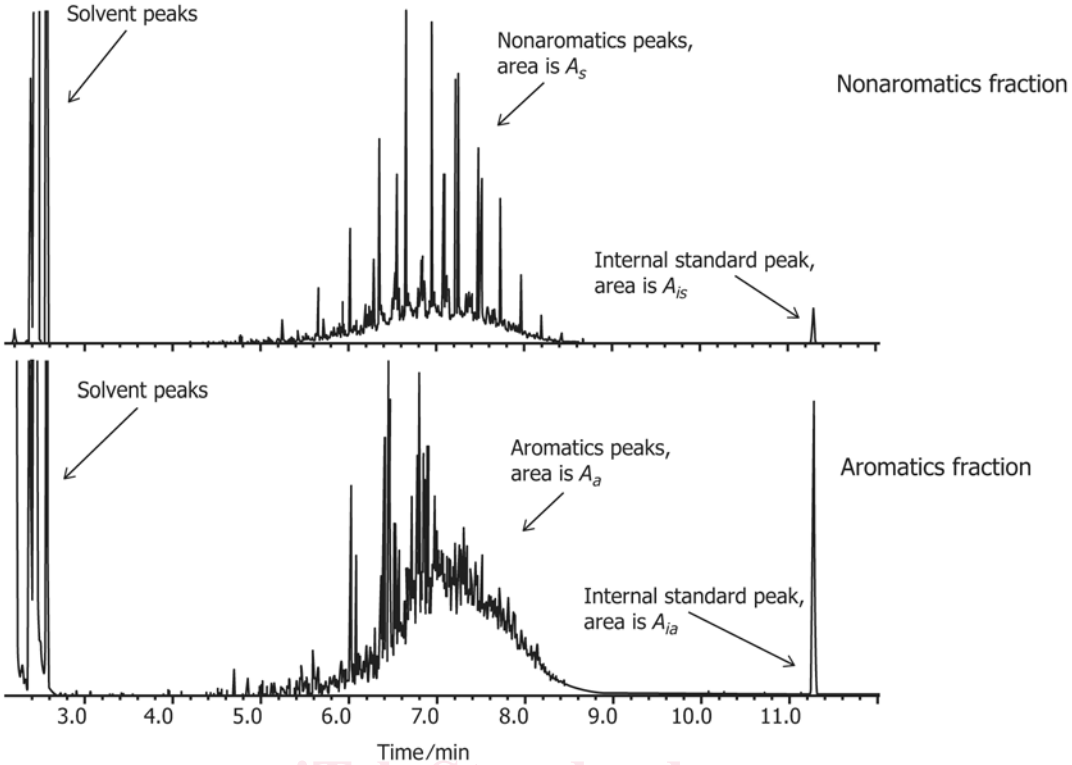

FIG. 2 Chromatograms of Nonaromatics and Aromatics Fractions in Petroleum-based Middle Distillate Fuel

7. Reagents and Materials

7.1 *Purity of Reagents*—Reagent grade chemicals shall be used in this test. 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,[5] where such specifications are available. 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.

7.2 *Ethyl Alcohol,* anhydrous, reagent grade. (**Warning—** Flammable.)

7.3 *Dichloromethane,* reagent grade. (**Warning—**Toxic. Harmful if inhaled or skin contact.)

7.4 *n-hexane,* reagent grade. (**Warning—**Flammable.)

7.5 *n-hexadecane,* reagent grade.

7.6 *n-triacontane,* reagent grade.

7.7 *n-dotriacontane,* reagent grade.

7.8 *Methyl oleate,* >99 %.

7.9 *Internal Standard Solution,* n-triacontane or n-dotriacontane dissolved in n-hexane solvent to obtain the mass concentrations of 0.001 g/mL to 0.005 g/mL.

7.10 *Silica Gel,* 75 µm to 150 µm.

7.11 *Aluminum oxide (neutral),* 75 µm to 150 µm.

7.12 *Carrier gas,* Nitrogen, Helium, or Hydrogen, 99.99 % pure. (**Warning—**Compressed gas under high pressure.)

7.13 *Hydrogen,* 99.9 % pure. (**Warning—**Extremely flammable gas under high pressure.)

7.14 *Air,* compressed, 99.9% pure. (**Warning—**Compressed gas under high pressure that supports combustion.)

7.15 *Column Fractionation Efficiency Evaluation Mixture (Procedure A),* used to evaluate the separation efficiency of SPE column in Procedure A. The mixture can be prepared by pure saturates in the range of 50 % to 95 % by mass and pure aromatics in the range of 5 % to 50 % by mass. The pure saturates can be a qualitative mixture of at least three paraffins from n-C_{10} to n-C_{20}.The pure aromatics can be a qualitative mixture of at least three alkylbenzenes, such as C_4 to C_{12}-benzene, naphthalene, and phenanthrene. It is recommended that the concentrations of alkylbenzene and polycyclic aromatics are similar with that in a diesel fuel sample. The representative compositions and concentrations for the evaluation mixtures are listed in Table A1.1 in Annex A1.

7.16 *Column Fractionation Efficiency Evaluation Mixture (Procedure B),* used to evaluate the separation efficiency of SPE column in Procedure B. The mixture can be prepared by pure saturates in the range of 30 % to 95 % by mass, pure aromatics in the range of 5 % to 50 % by mass and FAME in the range of 0.5 % to 20 % by volume. The pure saturates can be a qualitative mixture of at least three paraffins from n-C_{10} to

[5] *ACS Reagent Chemicals, Specifications and Procedures for Reagents and Standard-Grade Reference Materials*, 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.

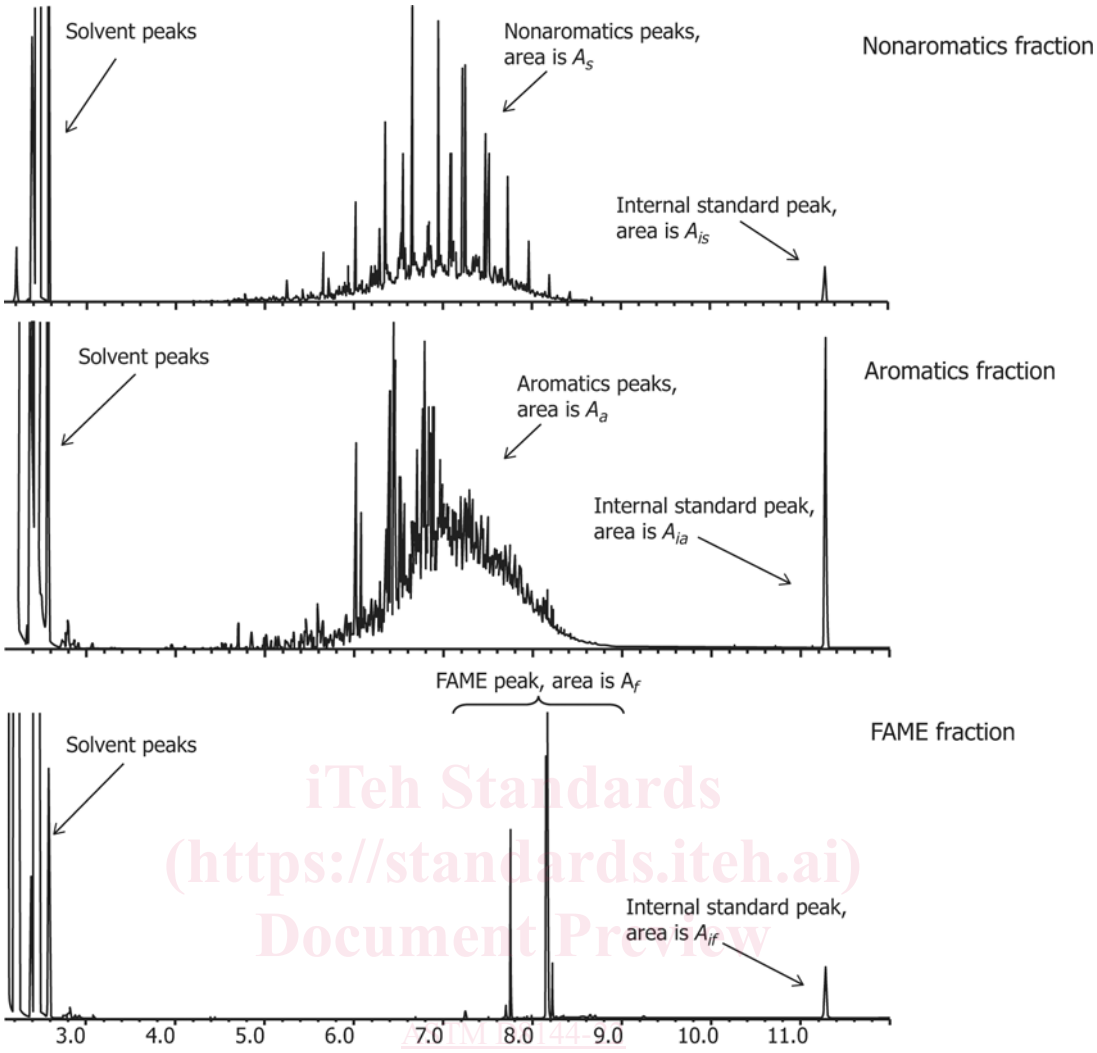

FIG. 3 Chromatograms of Nonaromatics, Aromatics, and FAME Fractions in FAME Biodiesel Blends

n-C_{20}. The pure aromatics can be a qualitative mixture of at least three alkylbenzenes, such as C_4 to C_{12}-benzene, naphthalene, and phenanthrene. It is recommended that the concentrations of alkylbenzene and polycyclic aromatics are similar with that in a diesel fuel sample. Any FAME standard or a mixture of FAME standards can be used to prepare this mixture. The representative composition and concentrations for the evaluation mixtures are listed in Table A1.2 in Annex A1.

7.17 *Relative Response Factor Mixture,* used to calculate the relative response factor of FAME (relative to n-hexadecane). Prepare a quantitative mixture of methyl oleate (see Note 4) and n-hexadecane at the weight ratio of 1:1. Use n-hexane as the solvent to provide a solution with a mass percent of 1 %.

NOTE 4—Any FAME standard or a mixture of FAME standard can be used as the reference to calculate the relative response factor.

7.18 *Quality Control Sample (Procedure A),* used to routinely monitor the operation of the gas chromatographic system and verify that the reported concentrations are within the precision of the test method. The quality control (QC) sample is prepared by weighing and blending a certain mass of aromatics and nonaromatics fractions, which are separated from Test Method D2549. The composition of QC sample shall be similar with that in test sample.

7.19 *Quality Control Sample (Procedure B),* used to routinely monitor the operation of the gas chromatographic system and verify that the reported concentration are within the precision of the test method. The quality control (QC) sample is prepared by weighing and blending a certain mass of FAME biodiesel with the aromatics and nonaromatics fractions, which are separated from Test Method D2549. The composition of QC sample shall be similar with that in test sample.

7.20 *Reference Sample of Middle Distillates,* middle distillates with known content of aromatics and nonaromatics fractions, a check standard meeting the requirement in Practice D6299 with accepted reference value (ARV) determined by Test Method D2549.

7.21 *Reference Sample blended with FAME,* diesel fuel with known content of aromatics, nonaromatics, and FAME, a

check standard prepared by spiking the weighed FAME into the reference sample listed in 7.20. The ARV shall be determined in accordance with their blended weight ratio.

8. Sampling

8.1 Unless otherwise specified, samples shall be obtained in accordance with Practices D4057, D4177, or other comparable practices. Samples should be stored in sealed containers.

9. Preparation of Apparatus

9.1 *Chromatograph*—Place in service and maintain in accordance with the manufacturer's instructions. Typical operating conditions are shown in Table 1, but manufacturer's recommendations should be followed when possible.

10. Standardization and Verification

10.1 *Verification of the Separation Efficiency of the SPE Column:*

10.1.1 For a new batch of SPE columns or a new stationary phase used in the SPE column, or when overall performance criteria are not being met, it is necessary to determine the separation efficiency of the column in accordance with the procedure as follows.

10.1.2 Use the separation efficiency evaluation mixture listed in 7.15 and 7.16 to evaluate the separation efficiency of the SPE fractionation column. The detailed procedure and calculation used in fractionation column evaluation are described in Annex A1. Representative chromatograms for the evaluation mixtures are shown in Fig. A1.1 and Fig. A1.2. Any SPE fractionation column that satisfies the separation efficiency requirements listed in Table 2 and Table 3 is acceptable.

NOTE 5—If this sample fractionation procedure is used as the preparation procedure for Test Method D2425, the SPE fractionation efficiency can be directly checked by the mass spectrometric analysis results of a petroleum middle distillate sample. The overlap between aromatics and nonaromatics fractions in a petroleum sample should be less than 5 %.

10.2 *Verification of the Performance of the Gas Chromatographic System:*

10.2.1 Analyze the QC sample listed in 7.18 and 7.19 in accordance with 11.1 and 11.2 to check the baseline separation of solvent, sample, and internal standards. If necessary, adjust the column temperature program to ensure their baseline separation. The quality control/quality assurance (QC/QA) protocols are recommended to be established based on Practice D6299 and MNL7.[6] The QC sample precision should be periodically checked against the site precision to ensure data quality (See Practice D6299 and MNL7). The site precision is expected to be better or equal to the published reproducibility.

[6] ASTM MNL 7 "Manual on Presentation of Data Control Chart Analysis," 8th ed., available from ASTM Headquarters.

TABLE 2 Requirements of Separation Efficiency for Nonaromatics and Aromatics Standards in Procedure A and B

Range of the mass percent of aromatics standards	Overlap between nonaromatics and aromatics (mass percent)
$10 \le x \le 50$	<5
$5 \le x < 10$	<10

TABLE 3 Requirements of Separation Efficiency for FAME and Hydrocarbons Standards in Procedure B

Range of the volume percent of FAME	Mass percent of hydrocarbons in FAME fraction	Mass percent of FAME in aromatics fraction
$1.0 \le x \le 20$	<5	<5
$0.5 \le x < 1.0$	<10	

10.3 *Verification for the Total Operation of the System:*

10.3.1 Analyze the reference sample listed in 7.20 and 7.21 routinely and the determined results of reference sample should agree with their accepted reference values (ARVs). If the test result is not within $\pm$ (R/$\sqrt{2}$) of the ARV, in which R is the reproducibility of the test method, check the separation efficiency of the SPE column and the integration results of chromatographic peaks. Reanalyze the reference sample until they meet this criterion.

11. Procedure A and B

11.1 *Procedure A: Analysis of Petroleum-based Middle Distillate Fuels:*

11.1.1 *Fractionation Procedure using Solid Phase Extraction (SPE):*

11.1.1.1 Add 0.5 mL n-hexane to the top of the SPE column to pre-wet the adsorbent.

11.1.1.2 Add 0.1 mL sample to the top of the column and wait until it is completely adsorbed.

11.1.1.3 Place a 10 mL Erlenmeyer flask beneath the column to collect the eluate. Sequentially add 2 mL n-hexane and then 0.5 mL dichloromethane-ethyl alcohol mixture (50 to 10 by volume) to the top of the column to elute the nonaromatics fraction. The flow rate should be approximately 2 mL/min. Label the 10 mL flask as nonaromatics fraction.

NOTE 6—Pressure can be applied to the top of the SPE adaptor by an air-filled syringe to maintain the elution rate at approximately 2 mL/min.

11.1.1.4 When the meniscus of the dichloromethane-ethyl alcohol eluent has reached the top of the packing bed, change the receiver to another 10 mL Erlenmeyer flask. Rinse the column with 2 mL dichloromethane-ethyl alcohol mixture (50 to 10 by volume) to elute the aromatics fraction. The flow rate should be approximately 2 mL/min. Label the 10 mL flask as aromatics fraction (see Note 6).

11.1.1.5 Quantitatively add 1 mL internal standard solution to the two flasks containing nonaromatics and aromatics fractions respectively and mix well.

11.1.2 *Gas Chromatography (GC) Analysis:*

11.1.2.1 *Instrument Preparation*—Perform a blank analysis to ensure that there is no carryover from previous sample analysis.

NOTE 7—Generally, a chromatogram which has no peaks with an S/N >30 is required for a suitable blank run. If carry-over is observed in a blank run, a 30 min column conditioning at 300 °C is recommended to remove the residual samples, after which another blank run should be done to verify success.

11.1.2.2 Inject and analyze 0.5 µL of each fraction prepared in 11.1.1.5, Fig. 2 illustrates representative chromatograms and relative peak sizes of aromatics, nonaromatics fractions as well as internal standards.

NOTE 8—Check the integration results to ensure all of the peaks are integrated adequately (start/stop times, baselines). If necessary, change the parameters of automated integrator or integrate peaks manually to ensure accuracy.

11.2 *Procedure B: Analysis of FAME Biodiesel Blends:*

11.2.1 *Fractionation Procedure using Solid Phase Extraction (SPE):*

11.2.1.1 Add 0.5 mL n-hexane to the top of the SPE column to pre-wet the adsorbent.

11.2.1.2 Add 0.1 mL sample to the top of the column and wait until it is completely adsorbed.

11.2.1.3 Place a 10 mL Erlenmeyer flask beneath the column to collect the eluate. Sequentially add 2 mL n-hexane and then 0.5 mL dichloromethane-n-hexane mixture (50 to 50 by volume) to the top of the column to elute the nonaromatics fraction. The flow rate should be approximately 2 mL/min. Label the 10 mL flask as nonaromatics fraction.

NOTE 9—Pressure can be applied to the top of the SPE adaptor by an air-filled syringe to maintain the elution rate at approximately 2 mL/min.

11.2.1.4 When the meniscus of the dichloromethane-n-hexane eluent has reached the top of the packing bed, change the receiver to another 10 mL Erlenmeyer flask. Rinse the column sequentially with an additional 2 mL dichloromethane-n-hexane mixture and then 0.5 mL dichloromethane-ethyl alcohol mixture (50 to 50 by volume) to elute the aromatics fraction. The flow rate should be approximately 2 mL/min. Label the 10 mL flask as aromatics fraction (see Note 9).

11.2.1.5 When the meniscus of the dichloromethane-ethyl alcohol mixture level reaches the top of the packing bed, change the receiver to a third 10 mL Erlenmeyer flask. Rinse the column with 6 mL dichloromethane-ethyl alcohol mixture (50 to 50 by volume) to elute the FAME fraction. The flow rate should be approximately 2 mL/min (see Note 9). Label the 10 mL flask as FAME fraction.

11.2.1.6 Quantitatively add 1 mL internal standard solution to each of the three flasks containing nonaromatics, aromatics, and FAME fractions and mix well.

11.2.2 *Gas Chromatography (GC) Analysis:*

11.2.2.1 *Instrument Preparation*—Perform a blank analysis to ensure that there is no carryover from previous sample analysis.

NOTE 10—Generally, a chromatogram no peak with S/N >30 is required for a successful blank run. If carry-over is observed in a blank run, a 30 min column conditioning at 300 °C is required to remove the residual samples followed by another blank run to verify success.

11.2.2.2 Inject and analyze 0.5 µL of the relative response factor mixture prepared in 7.17. The relative response factor can be calculated according to the weights of methyl oleate and n-hexadecane.

11.2.2.3 Inject and analyze 0.5 µL of each fraction prepared in 11.2.1.6. Fig. 3 illustrates representative chromatograms and relative peak sizes of nonaromatics, aromatics, FAME fractions as well as internal standards.

NOTE 11—Check the integration results to ensure all of the peaks are integrated adequately (start/stop times, baselines). If necessary, change the automated integration parameters or integrate peaks manually.

12. Calculation

12.1 *Equations Applied to Procedure A:*

12.1.1 Calculate the mass percent of the aromatics fraction in middle distillates according to Eq 1.

$$X_1 = \frac{A_a / A_{ia}}{A_s / A_{is} + A_a / A_{ia}} \times 100 \quad (1)$$

12.1.2 Calculate the mass percent of the nonaromatics fraction in middle distillates according to Eq 2.

$$X_2 = \frac{A_s / A_{is}}{A_s / A_{is} + A_a / A_{ia}} \times 100 \quad (2)$$

where:

X_1 = mass percent of the aromatics fraction in middle distillates,
X_2 = mass percent of the nonaromatics fraction in middle distillates,
A_a = total peak area of the aromatics in chromatogram of aromatics fraction,
A_{ia} = peak area of the internal standard in chromatogram of aromatics fraction,
A_s = total peak area of the nonaromatics in chromatogram of nonaromatics fraction, and
A_{is} = peak area of the internal standard in chromatogram of nonaromatics fraction.

12.2 *Equations Applied to Procedure B:*

12.2.1 Calculate the relative response factor of FAME (relative to n-hexadecane) according to Eq 3.

$$B = \frac{w_1 / w_2}{S_F / S_{N16}} \quad (3)$$

where:

B = relative response factor of FAME,
w_1 = weight of methyl oleate in the mixture,
w_2 = weight of n-hexadecane in the mixture,
S_F = peak area of methyl oleate in the mixture, and
S_{N16} = peak area of n-hexadecane in the mixture.

12.2.2 Calculate the mass percent of the aromatics fraction in FAME biodiesel blends according to Eq 4.

$$X_3 = \frac{A_a / A_{ia}}{A_s / A_{is} + A_a / A_{ia} + (A_f \times B) / A_{if}} \times 100 \quad (4)$$

12.2.3 Calculate the mass percent of the nonaromatics fraction in FAME biodiesel blends according to Eq 5.

$$X_4 = \frac{A_s / A_{is}}{A_s / A_{is} + A_a / A_{ia} + (A_f \times B) / A_{if}} \times 100 \quad (5)$$

12.2.4 Calculate the mass percent of the FAME fraction in FAME biodiesel blends according to Eq 6.

$$X_5 = \frac{(A_f \times B) / A_{if}}{A_s / A_{is} + A_a / A_{ia} + (A_f \times B) / A_{if}} \times 100 \quad (6)$$

where:

X_3 = mass percent of the aromatics fraction in FAME biodiesel blends,
X_4 = mass percent of the nonaromatics fraction in FAME biodiesel blends,