



Designation: D5501 – 20

Standard Test Method for Determination of Ethanol and Methanol Content in Fuels Containing Greater than 20 % Ethanol by Gas Chromatography¹

This standard is issued under the fixed designation D5501; 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 ethanol content of hydrocarbon blends containing greater than 20 % ethanol. This method is applicable to denatured fuel ethanol, ethanol fuel blends, and mid-level ethanol blends.

1.1.1 Ethanol is determined from 20 % by mass to 100 % by mass and methanol is determined from 0.01 % by mass to 0.6 % by mass. Equations used to convert these individual alcohols from percent by mass to percent by volume are provided.

NOTE 1—Fuels containing less than 20 % ethanol may be quantified using Test Method D5599, and less than 12 % ethanol may be quantified using Test Method D4815.

1.2 This test method does not purport to identify all individual components common to ethanol production or those components that make up the denaturant or hydrocarbon constituent of the fuel.

1.3 Water cannot be determined by this test method and shall be measured by a procedure such as Test Method D7923, E203, or E1064 and the result used to correct the concentrations determined by this method.

1.4 This test method is inappropriate for impurities that boil at temperatures higher than 225 °C or for impurities that cause poor or no response in a flame ionization detector, such as water.

1.5 The values stated in SI units are to be regarded as the standard. The values given in parentheses are provided for information purposes only.

1.6 *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 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 Hydrocarbon Analysis.

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1.7 *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

- D1298 Test Method for Density, Relative Density, or API Gravity of Crude Petroleum and Liquid Petroleum Products by Hydrometer Method
- D1364 Test Method for Water in Volatile Solvents (Karl Fischer Reagent Titration 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
- D4175 Terminology Relating to Petroleum Products, Liquid Fuels, and Lubricants
- D4307 Practice for Preparation of Liquid Blends for Use as Analytical Standards
- D4626 Practice for Calculation of Gas Chromatographic Response Factors
- D4806 Specification for Denatured Fuel Ethanol for Blending with Gasolines for Use as Automotive Spark-Ignition Engine Fuel
- D4815 Test Method for Determination of MTBE, ETBE, TAME, DIPE, tertiary-Amyl Alcohol and C₁ to C₄ Alcohols in Gasoline by Gas Chromatography
- D5599 Test Method for Determination of Oxygenates in Gasoline by Gas Chromatography and Oxygen Selective Flame Ionization Detection
- D5798 Specification for Ethanol Fuel Blends for Flexible-Fuel Automotive Spark-Ignition Engines
- D6299 Practice for Applying Statistical Quality Assurance

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

and Control Charting Techniques to Evaluate Analytical Measurement System Performance

D6792 Practice for Quality Management Systems in Petroleum Products, Liquid Fuels, and Lubricants Testing Laboratories

D7923 Test Method for Water in Ethanol and Hydrocarbon Blends by Karl Fischer Titration

E203 Test Method for Water Using Volumetric Karl Fischer Titration

E355 Practice for Gas Chromatography Terms and Relationships

E594 Practice for Testing Flame Ionization Detectors Used in Gas or Supercritical Fluid Chromatography

E1064 Test Method for Water in Organic Liquids by Coulometric Karl Fischer Titration

E1510 Practice for Installing Fused Silica Open Tubular Capillary Columns in Gas Chromatographs

E3237 Specification for Undenatured Ethanol from Biomass for Use in Industrial Applications

2.2 *U.S. Federal Standards*.³

40 CFR 80.1426 How are RINs generated and assigned to batches of renewable fuel by renewable fuel producers or importers?

27 CFR 30.22 Hydrometers and Thermometers

27 CFR 30.67 Table 7, for correction of volume of spirituous liquors to 60 °F

3. Terminology

3.1 *Definitions*—This test method makes reference to many common chromatographic procedures, terms, and relationships. Detailed definitions can be found in Terminology **D4175**, and Practices **E355** and **E594**.

3.2 *Definitions*:

3.2.1 *denatured fuel ethanol, n*—fuel ethanol made unfit for beverage use by the addition of denaturants under formula(s) approved by the applicable regulatory agency to prevent the imposition of beverage alcohol tax.

3.2.2 *mass response factor (MRF), n*—constant of proportionality that converts area to mass percent.

3.2.3 *relative mass response factor (RMRF), n*—mass response factor of a component divided by that of another component.

3.2.3.1 *Discussion*—In this test method, the mass response factors are relative to that of n-heptane.

3.2.4 *tangential skimming, n*—in gas chromatography, integration technique used when a “rider” peak elutes on the tail of a primary peak.

3.2.4.1 *Discussion*—Since the majority of the area beneath the rider peak belongs to the primary peak, in tangential skimming the top of the primary peak tail is used as the baseline of the rider peak, and the triangulated area beneath the rider peak is added to the primary peak.

3.3 *Abbreviations*:

3.3.1 *CFR*—U.S. Code of Federal Regulations

3.3.2 *EPA*—The U.S. Environmental Protection Agency

3.3.3 *MRF*—mass response factor

3.3.4 *RIN*—Renewable Identification Number

3.3.5 *RMRF*—relative mass response factor

3.3.6 *TTB*—The Alcohol and Tobacco Tax and Trade Bureau of the U.S. Department of Treasury

4. Summary of Test Method

4.1 A representative aliquot of the fuel ethanol sample is introduced into a gas chromatograph equipped with a polydimethylsiloxane bonded phase capillary column. Carrier gas transports the vaporized aliquot through the column where the components are chromatographically separated in order of boiling point temperature. Components are sensed by a flame ionization detector as they elute from the column. The detector signal is processed by an electronic data acquisition system. The ethanol and methanol components are identified by comparing their retention times to the ones identified by analyzing standards under identical conditions. The concentrations of all components are determined in mass percent by normalization of the peak areas. After correction for water content, results may be reported in mass percent or volume percent.

5. Significance and Use

5.1 This test method provides a method of determining the percentage of ethanol in an ethanol-gasoline fuel blend over the range of 20 % by mass to 100 % by mass for compliance with fuel specifications and federal or local fuel regulations.

5.2 Ethanol content of denatured fuel ethanol for gasoline blending is required in accordance with Specification **D4806**.

5.3 Ethanol content of ethanol fuel blends for flexible-fuel automotive spark-ignition engines is required in accordance with Specification **D5798**.

5.4 This test method is acceptable for determining the percentage of ethanol for either denatured fuel ethanol or undenatured ethanol for use in industrial applications using Table 3(a) or Table 3(b).

5.4.1 Refer to local, federal, or other authorities having jurisdiction for information regarding the correct implementation of either Table 3(a) or Table 3(b).

5.4.2 Specific regulatory requirements for U.S. domestic fuel applications are given in **Appendix X5** for information.

6. Apparatus

6.1 *Gas Chromatograph*, capable of operating at the conditions listed in **Table 1**. A heated flash vaporizing injector designed to provide a linear sample split injection (for example, 200:1) is required for proper sample introduction. Carrier gas controls shall be of adequate precision to provide reproducible column flows and split ratios in order to maintain analytical integrity. Pressure and flow control devices shall be designed to attain the linear velocity required in the column used. A hydrogen flame ionization detector with associated gas controls and electronics, designed for optimum response with open tubular columns, is required.

³ Available from U.S. Government Printing Office, Superintendent of Documents, 732 N. Capitol St., NW, Washington, DC 20401-0001, <http://www.access.gpo.gov>.

TABLE 1 Typical Operating Conditions

Column Temperature Program		
Column length	100 m	150 m
Initial temperature	15 °C	60 °C
Initial hold time	12 min	15 min
Program rate	30 °C/min	30 °C/min
Final temperature	250 °C	250 °C
Final hold time	19 min	23 min
Injector		
Temperature	300 °C	
Split ratio	200:1	
Sample size	0.1 µL to 0.5 µL	
Detector		
Type	Flame ionization	
Temperature	300 °C	
Fuel gas	Hydrogen (30 mL/min)	
Oxidizing gas	Air (300 mL/min)	
Make-up gas	Helium or Nitrogen (30 mL/min)	
Date rate	20 Hz	
Carrier Gas		
Type	Helium or Hydrogen ⁴	
Average linear velocity	21 cm/s to 24 cm/s (constant flow)	

⁴ Use of hydrogen carrier gas requires additional safety considerations.

6.2 *Sample Introduction*—Automatic liquid syringe sample injection to the splitting injector. Devices capable of 0.1 µL to 0.5 µL injections are suitable.

NOTE 2—Inadequate splitter, poor injection technique, and overloading the column can result in poor resolution. Avoid overloading, particularly of the ethanol peak, and eliminate this condition during analysis.

6.3 *Column*—The precision for this test method was developed utilizing a fused silica open tubular column with non-polar polydimethylsiloxane bonded (cross-linked) phase internal coating. Any column with equivalent or better chromatographic efficiency, resolution, and selectivity to those described in 6.3.1 may be used.

6.3.1 Open tubular column with a non-polar polydimethylsiloxane bonded (cross-linked) phase internal coating, either 150 m by 0.25 mm with a 1.0 µm film thickness, or 100 m by 0.25 mm with a 0.5 film thickness have been found suitable. The 150 m column is recommended due to its higher resolution. Follow Practice E1510 for column installation.

6.4 *Electronic Data Acquisition System*—Any data acquisition and integration device used for quantification of these analyses must meet or exceed these minimum requirements:

- 6.4.1 Capacity for at least 80 peaks/analysis,
- 6.4.2 Normalized percent calculation based on peak area and using response factors,
- 6.4.3 Identification of individual components based on retention time,
- 6.4.4 Noise and spike rejection capability,
- 6.4.5 Sampling rate for narrow (<1 s) peaks,
- 6.4.6 Positive and negative sloping baseline correction,
- 6.4.7 Peak detection sensitivity compensation for narrow and broad peaks, and
- 6.4.8 Capable of integrating non-resolved peaks by perpendicular drop or tangential skimming as needed.

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

7.2 *Carrier Gas*, helium or hydrogen, with a minimum purity of 99.95 mol%. Oxygen removal systems and gas purifiers should be used. (**Warning**—Compressed gas under high pressure.) Use of hydrogen carrier gas may require additional safety considerations.

7.3 *Detector Gases*, hydrogen, air, and nitrogen. The minimum purity of the gases used should be 99.95 % for the hydrogen and nitrogen. The air should be hydrocarbon-free grade. Gas purifiers are recommended for the detector gases. (**Warning**—Hydrogen, extremely flammable gas under high pressure.) (**Warning**—Air and nitrogen, compressed gases under high pressure.)

7.4 *Standards for Calibration and Identification*—Standards of all components to be analyzed are required for establishing identification by retention time as well as calibration for quantitative measurements. These materials shall be of known purity and free of the other components to be analyzed.

7.4.1 To verify the purity of blend components, analyze each compound by the same technique for which the blend is intended or by another suitable technique.

7.4.2 Check for other impurities such as water. Water cannot be determined with sufficient accuracy by most GC methods and shall be measured by other procedures such as Test Method D1364, or equivalent, and the result used to normalize the chromatographic value. If any of the impurities found are components present in the blend, determine their concentrations and make appropriate corrections.

7.4.3 *Ethanol*—Absolute ethanol, 99.5 % by mass minimum. Methanol content should be less than 0.01 % by mass. (**Warning**—Flammable and may be harmful or fatal if ingested or inhaled.)

7.4.4 *Methanol*—(**Warning**—Flammable and may be harmful or fatal, if ingested or inhaled.) Minimum purity of 99 % by mass, and free of ethanol.

7.4.5 *Heptane*—(**Warning**—Flammable and may be harmful or fatal, if ingested or inhaled.)

7.4.6 *Hydrocarbon Diluent*—n-Octane or isooctane, used for preparation of calibration standards. The diluent shall contain <0.01 % of heptane, methanol, or ethanol. (**Warning**—Flammable and may be harmful or fatal if ingested or inhaled.)

7.5 *Linearity Mixture*—A mixture of known composition containing 10-20 hydrocarbons, ranging from C5 to C11, used for split injector linearity testing. (**Warning**—Flammable and may be harmful or fatal if ingested or inhaled.)

8. Sampling

8.1 Denatured ethanol may be sampled into an open container since a vapor pressure of less than 21 kPa (3 psi) is expected. Refer to Practice D4057 for instruction on manual

⁴ 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.

sampling from bulk storage into open containers. Stopper the container immediately after drawing the sample.

8.2 To minimize loss of hydrocarbon light ends, chill the sample before transferring an aliquot into an auto sampler vial. Seal immediately. Obtain the test sample for analysis directly from the sealed auto sampler vial.

9. Preparation and Verification of Apparatus

9.1 Install and condition the column in accordance with the manufacturer's or supplier's instructions. After conditioning, attach column outlet to flame ionization detector inlet and check for leaks throughout the system. When leaks are found, tighten or replace fittings before proceeding.

9.2 Adjust the carrier gas flow rate so that the average linear gas velocity, at the initial temperature of the run, is between 21 cm/s and 24 cm/s, as determined by Eq 1. Flow rate adjustment is made by raising or lowering the carrier gas pressure (head pressure) to the injector. Maintain constant flow throughout the analysis.

$$\bar{\mu} = \frac{L}{t_m} \quad (1)$$

where:

$\bar{\mu}$ = average linear gas velocity (cm/s),

L = column length (cm), and

t_m = retention time of methane.

9.3 Adjust the operating conditions of the gas chromatograph (Table 1) and allow the system to equilibrate.

9.4 *Linearity*—The linearity of the gas chromatograph system shall be established prior to the analysis of samples.

9.4.1 The optimal split ratio is dependent upon the split linearity characteristics of the particular injector and the sample capacity of the column. The capacity of a particular column for a sample component is dependent on the amount and polarity of the liquid phase (loading or film thickness) and the ratio of the column temperature to the component boiling point (vapor pressure). Overloading of the column can cause loss of resolution for some components and, since overloaded peaks are skewed, variance in retention times. This can lead to erroneous component identification. During column evaluations and split linearity studies, be aware of any peaks that appear front skewed, indicating column overload. Note the component size and avoid conditions leading to this problem during actual analysis. Refer to Practice E594 for further guidance.

9.4.2 The injector split linearity is dependent on the nature of the compound (boiling point, molecular weight, etc.) splitter and liner design, injection volume, and the linear velocity of sample through the inlet. Establish the splitting injector linearity to determine the proper quantitative parameters and limits. Analyze the linearity mixture, 7.5, following the gas chromatographic analysis procedure in Section 12. Calculate the normalized mass percent (using Eq 8, Eq 5, and Eq 6) of each component using a relative mass response factor of 1 for all hydrocarbon compounds. The determined mass percent for each component shall match the gravimetric known concentration within $\pm 3\%$ relative.

9.4.3 Verify the linearity of the flame ionization detector (FID). Refer to Practice E594 for a suggested procedure. A plot of the peak areas versus concentration for prepared methanol or ethanol standards in the concentration range of interest should be linear. If the plot is not linear or does not conform to these requirements then adjust the split ratio.

9.5 Resolution and Integration:

9.5.1 Optimize the system to resolve methanol from isobutane on the 150 m column using the conditions in this method. With higher amounts of isobutane, the possibility for coelution increases. Improved resolution has been achieved by optimizing the linear velocity to 24 cm/s. See Fig. 1.

9.5.2 Methanol might not be completely resolved from Butene-1/Isobutylene on the 100 m column using the conditions in this method. Use tangential skimming to properly integrate the methanol.

9.5.3 Ethanol might not be completely resolved from 3-methyl-1-butene. If so, use tangential skimming to integrate the ethanol. See Fig. 2.

9.5.4 Set integration parameters such that any component of at least 0.002 % by mass is integrated.

9.6 Peak Shape:

9.6.1 System overload of ethanol can be determined upon visual inspection of the peak. A flat top indicates electronic saturation. A wide "fin" shape can indicate column overload. In either case, investigate and make adjustments.

9.6.2 Hydrocarbon peaks shall be symmetrical. Slight tailing of polar compounds (methanol, ethanol) is typical, but excessive tailing should be investigated. Verify that the inlet liners are thoroughly deactivated and the column shows appropriate deactivation towards methanol and ethanol. Fig. 3 shows an example of poor chromatography.

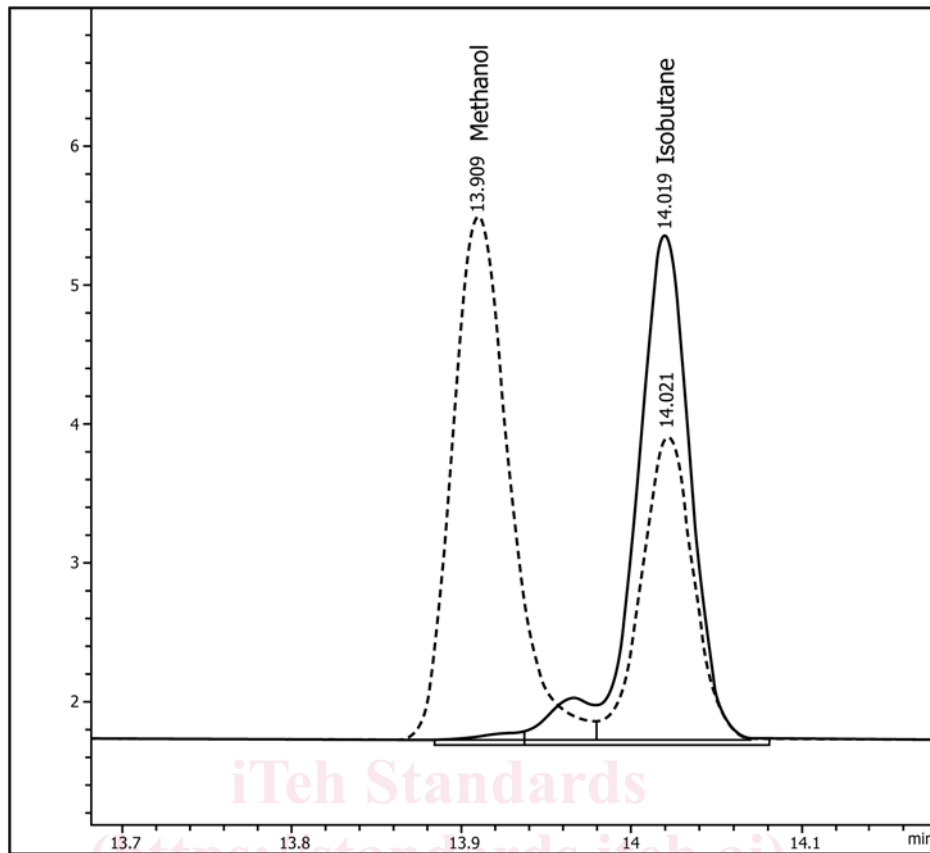


FIG. 1 Chromatogram Overlay Showing Possible Methanol Interferences on 150 m Column

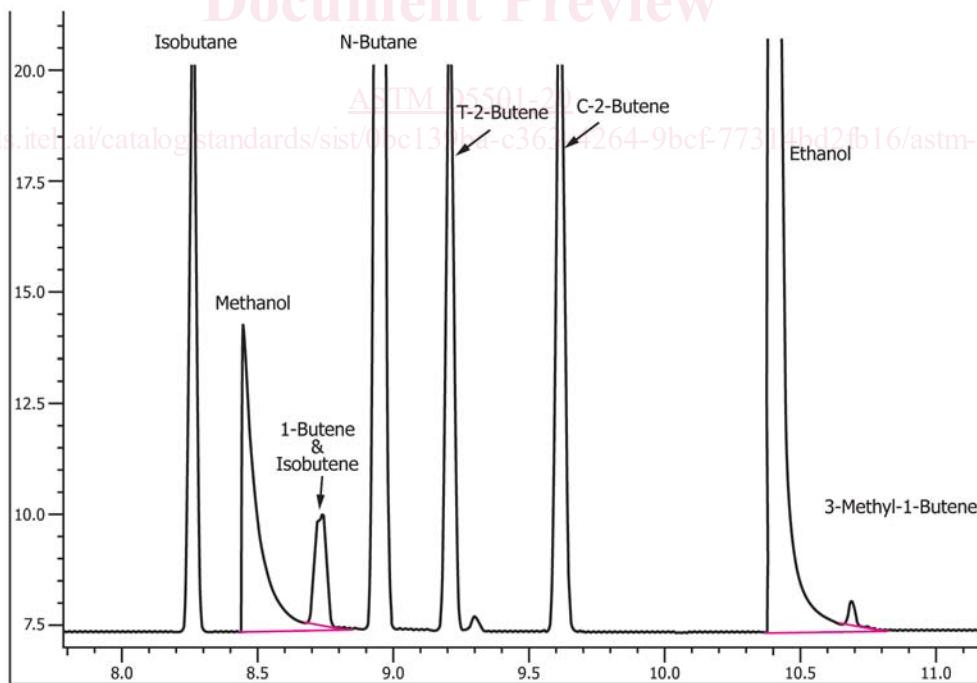
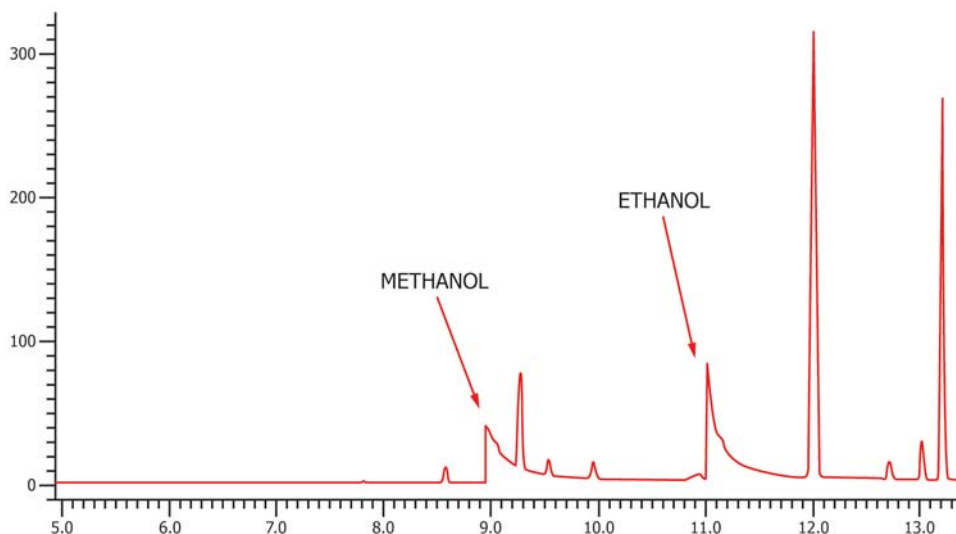


FIG. 2 Integration of Methanol and Ethanol on 100 m Column Using Tangential Skimming



NOTE 1—Excessive tailing and delayed elution may result from adsorption, dead volume, or damaged column.

FIG. 3 Example of Poor Chromatography

10. Calibration and Standardization

10.1 *Identification*—Determine the retention time of ethanol and methanol by injecting amounts of each, either separately or in known mixtures, in concentrations expected in the final blend.

10.2 Calibration Standards:

10.2.1 Determine the purity of the oxygenate reagents and make corrections for the impurities found. Whenever possible, use stock chemicals of at least 99.5 % purity. Correct the purity of the components for water content, determined by Test Method D1364 or equivalent. Example calculations are presented in Appendix X1.

10.2.2 Gravimetrically prepare standards that are blended according to Practice D4307. Chill components prior to blending. Prepare standards that cover the expected range of ethanol and methanol in a hydrocarbon diluent (n-octane or isooctane) and containing a known amount of heptane. Examples of standards covering the range of 20 % by mass to 99 % by mass ethanol are given in Table 2.

10.3 *Calibration Linearity*—Analyze the standards from 10.2 according to the procedure in Section 12. An example chromatogram is found in Fig. 4. For ethanol, methanol, and n-heptane, the plot of the peak areas versus concentration shall be linear with a minimum r^2 of 0.995. Do not force the ethanol calibration through the origin (zero). For ethanol, a peak area of zero shall correspond to an ethanol concentration between

TABLE 2 Recommended Matrix of Calibration Standards Ranging from 20 % to 99 % by Mass

	Mix 1	Mix 2	Mix 3	Mix 4	Mix 5
Methanol, % by mass	0.6	0.5	0.3	0.2	0.1
Ethanol, % by mass	20.0	50.0	75.0	90.0	99.4
Heptane, % by mass	10.0	10.0	10.0	4.0	0.5
Hydrocarbon diluent, ^a % by mass	69.4	39.5	14.8	5.8	0.0

^aHydrocarbon diluent is free of heptane and any other compounds that would interfere with the calibration. See 7.4.

–3 % and +3 % by mass (see Note 3). If the plot does not conform to these requirements, troubleshoot the system and repeat the analyses using fresh vials of standard until requirements are met. Typical troubleshooting measures include increasing the split ratio, reducing the injection amount, correcting the integration of the ethanol and methanol peaks, and checking for active sites in column and inlet liner. Example linearity plots for ethanol are given in Fig. 5.

10.4 *Calibration*—Calibration techniques may be classified as absolute or relative, and are typically dictated by the chromatographic software. Only after linearity is established, calibrate with one of the techniques below.

10.4.1 *Absolute Calibration*—Establish the correction factor or equation that directly relates the peak area to the component concentration. If available, multi-point calibration is preferred.

10.4.1.1 *Single-point*—Calculate the mass response factor (MRF) of ethanol in each calibration standard using Eq 2 according to Practice D4626. The average response factor determine at each concentration shall be used in the calibration. Repeat for methanol and heptane. Assign the heptane response factor to any unknowns.

$$MRF_{(i)} = \text{mass}\%_{(i)} / \text{area}_{(i)} \quad (2)$$

where:

$MRF_{(i)}$ = the mass response factor of component i,
 $Mass\%_{(i)}$ = the mass percent of component i, and
 $Area_{(i)}$ = the peak area of component i.

10.4.1.2 *Multi-point*—With this technique, calibration and linearity verification may be completed in the same step. Analyze the standards prepared in 10.2, according to the procedure in Section 12. Generate individual calibration curves for methanol, ethanol, and heptane following the software manufacturer’s instructions. Calibration curves shall be linear with a minimum r^2 of 0.995. Verify that the calibration curve of ethanol passes near the origin (see Note 3), but do not force the origin. Force methanol and heptane calibrations through zero if necessary to quantify small peaks. Failure to quantify small

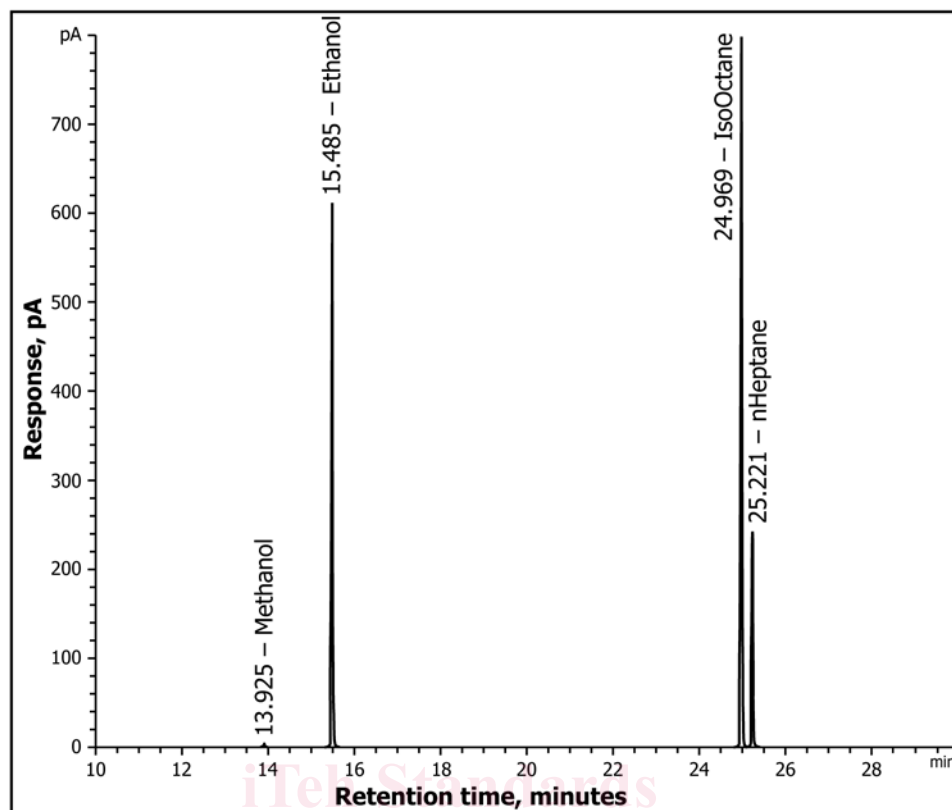


FIG. 4 Sample Chromatogram of Calibration Mixture on 150 m Column

unknown peaks will result in incorrect ethanol determination. The calibration equation of heptane shall be assigned to all unknowns.

NOTE 3—Software packages can differ in designation of the axes. If the peak area is on the x-axis, the y-intercept of the ethanol curve shall be between -3 % and +3 % by mass. If the peak area is on the y-axis, solving the calibration equation for $y = 0$ shall give a result between -3 % and +3 % by mass.

10.4.2 *Relative Calibration*—Tabulate the mass response factors (MRF) of ethanol, methanol, and n-heptane according to Eq 2. Then calculate the relative mass response factor (RMRF) of methanol and ethanol relative to heptane with Eq 3. Typical relative mass response factors for the components of interest are found in Table 3(a) and (b).

$$RMRF_{(i)} = MRF_{(i)} / MRF_{(heptane)} \quad (3)$$

where:

- $RMRF_{(i)}$ = the relative mass response factor of component i relative to n-C₇,
- $MRF_{(i)}$ = the mass response factor of component i, and
- $MRF_{(heptane)}$ = the mass response factor of heptane.

Average the experimental ethanol relative mass response factors determined for each standard. Use the average RMRF as the calibration value. Repeat for methanol. Assign heptane and all unknowns an RMRF of 1.000.

10.5 After completing the calibration, calculate the composition of each calibration standard according to the procedure in Section 13. Verify that the normalized results are in agreement with theoretical ethanol values within ±0.5 % and with theoretical methanol values within ±0.05 %.