



# Standard Test Methods for Fatty and Rosin Acids in Tall Oil Fractionation Products by Capillary Gas Chromatography<sup>1</sup>

This standard is issued under the fixed designation D 5974; 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 ( $\epsilon$ ) indicates an editorial change since the last revision or reapproval.

## 1. Scope

1.1 These test methods cover the determination of the amounts of the individual fatty acids and rosin acids in fractionated tall oil products, using capillary gas chromatographic separation of the volatile methyl esters of these acids.

1.2 Four methods for forming the methyl esters, and two methods for determining the amounts of the individual fatty acids and rosin acids are described.

1.2.1 The classic method for the formation of methyl esters is through the use of diazomethane, but diazomethane is a hazardous and toxic material, and so is no longer the preferred reagent. The use of diazomethane is detailed in the Appendix. Methyl esters may be formed through the use of tetramethylammonium hydroxide (TMAH), trimethylphenylammonium hydroxide (TMPAH), or N,N-dimethylformamide dimethyl acetal (DMF-DMA).

1.2.2 The two methods for determining the amount of the individual fatty acids and rosin acids are the “internal standard” method, which yields absolute values, and the “area percent” method, which yields relative values.

1.3 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

## 2. Referenced Documents

### 2.1 ASTM Standards:

D 509 Test Methods of Sampling and Grading Rosin<sup>2</sup>

D 804 Terminology Relating to Naval Stores, Including Tall Oil and Related Products<sup>2</sup>

E 691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method<sup>3</sup>

<sup>1</sup> This test method is under the jurisdiction of ASTM Committee D01 on Paint and Related Coatings, Materials, and Applications and is the direct responsibility of Subcommittee D01.34 on Naval Stores.

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<sup>2</sup> *Annual Book of ASTM Standards*, Vol 06.03.

<sup>3</sup> *Annual Book of ASTM Standards*, Vol 14.02.

## 3. Significance and Use

3.1 Tall oil fractionated products derived from tall oil are important commercial materials, primarily composed of fatty acids and rosin acids, but also containing some neutral material (see Terminology D 804). For many applications, it is necessary to know the level of the individual fatty acids and rosin acids present in these products. Gas chromatography has proven to be a useful tool for such determinations (see Test Methods D 509), and capillary chromatography, described in these test methods, is considered to be the most effective gas chromatographic technique currently available. In particular situations, other techniques may be more suitable than gas chromatography. For example, the presence of fatty acid esters in the sample would result in transesterification during the derivatization step that may affect the results.

3.2 Due to hydrogen bonding, unmodified tall oil fatty acids and rosin acids cannot be volatilized at atmospheric pressure without undergoing decomposition. So, it is necessary to convert the free acids to the more volatile and more stable methyl esters, prior to chromatographic separation.

3.3 These test methods describe four ways to prepare methyl esters. The classic method is through the use of diazomethane, but diazomethane is a hazardous and toxic material, and so is no longer the preferred agent. The use of diazomethane is detailed in the Appendix.

3.3.1 TMAH causes isomerization of a sample's di- and polyunsaturated fatty acids, when it is used in even a slight excess. This leads to inaccurate results for the individual fatty acid components. TMAH should be used for materials containing only rosin acids, or when the identification or quantitation of individual fatty acid components is not important.

3.3.2 TMPAH is the recommended methylating agent when the identification or quantitation of individual di- and polyunsaturated fatty acids is required. TMPAH produces results that are very similar to those of diazomethane, but without the hazards that are associated with diazomethane. A considerable excess of TMPAH may cause isomerization of conjugated compounds similar to that encountered with TMAH.

3.3.3 DMF-DMA gives results comparable to TMPAH and is easy and safe to use. However, the reagent is moisture sensitive, requiring samples to be free of any significant levels of water.

3.4 Two test methods for calculating the amounts of the individual fatty acid and rosin acid methyl esters are included in these test methods. When the actual weight percentage of a given compound is required, the “internal standard” method must be used. This method involves adding a known amount of an internal standard to a known amount of test material, and comparing the area of the peak associated with the internal standard with the area of the peak of the individual fatty acid or rosin acid methyl esters. The “area percent” method will give the relative amount of each component, by comparing the area of the appropriate peak to the total area of all peaks. Non-eluting compounds will lead to erroneous (absolute) results with this method.

### PREPARATION OF METHYL ESTERS

NOTE 1—Any of these three methods can be used, with the choice being dependent on the factors mentioned in 3.3.

#### 4. Conversion By Means of Tetramethylammonium Hydroxide (TMAH)

##### 4.1 Apparatus:

##### 4.1.1 Standard Laboratory Equipment .

##### 4.2 Reagents and Materials:

4.2.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all 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<sup>4</sup>, 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.

4.2.2 *Tetramethylammonium Hydroxide Solution, 24 % in methanol, CAS No. 75-59-2.*

4.2.3 *Tetramethylammonium Hydroxide Solution, 6 % (v/v) in methanol. Dilute 25 mL of the reagent described in 4.2.2 with 75 mL of methanol.*

4.2.4 *Phenolphthalein Solution, 1 % (w/v) in methanol.*

4.2.5 *Diethyl Ether, anhydrous.*

4.2.6 *Methanol, anhydrous.*

4.2.7 *Acetic Acid, 5 % volume/volume (v/v) in methanol.*

4.2.8 *Toluene, optional.*

##### 4.3 Procedure:

4.3.1 Dissolve the sample from 9.2.2 or 17.1 in 0.5 to 3.0 mL of a 50:50 ether/methanol mixture, add 2 to 3 drops of phenolphthalein indicator solution, and titrate to a pH of 7.8 to 8.1 or to the very first permanent pink color, with the 6 % solution of TMAH. If the solution is overtitrated, it can be back titrated with the acetic acid in methanol solution to the end

point. When the solution is injected into the heated injection port of the chromatograph, the tetramethylammonium salts are pyrolyzed to methyl esters.

NOTE 2—For solid rosin, or other samples that are difficult to dissolve, 2 to 3 drops of toluene may be added to the vial prior to the addition of TMAH, to assist in dissolving the sample.

#### 5. Conversion By Means of Trimethylphenylammonium Hydroxide (TMPAH)

##### 5.1 Apparatus:

##### 5.1.1 Standard Laboratory Equipment.

##### 5.2 Reagents and Materials:

5.2.1 *Purity of Reagents, see 4.2.1.*

5.2.2 *Trimethylphenylammonium Hydroxide Solution, 0.2 M or 0.1 M in methanol, CAS No. 1899-02-1.*

5.2.3 *Diethyl Ether, anhydrous.*

5.2.4 *Methanol, anhydrous.*

5.2.5 *Toluene, optional*

##### 5.3 Procedure:

5.3.1 Add 0.5 to 3.0 mL of a 50:50 ether/methanol, to the sample from 9.2.2 or 17.1. Add 2 to 3 drops of phenolphthalein indicator solution and titrate to the very first permanent pink color with the TMPAH in methanol solution. When the solution is injected into the heated injection port of the chromatograph, the trimethylphenylammonium salts are pyrolyzed to their respective methyl esters.

NOTE 3—For solid rosin, or other samples that are difficult to dissolve, 2 to 3 drops of toluene may be added to the vial prior to the addition of TMPAH, to assist in dissolving the sample.

#### 6. Conversion by Means of N,N-Dimethylformamide Dimethyl Acetal (DMF-DMA).

##### 6.1 Apparatus:

##### 6.1.1 Standard Laboratory Equipment .

##### 6.2 Reagents and Materials:

6.2.1 *Purity of Reagents, see 4.2.1.*

6.2.2 *N,N-Dimethylformamide dimethyl acetal (DMF-DMA), CAS No. 4637-24-5.*

6.2.3 *Methanol, anhydrous.*

6.2.4 *Toluene.*

##### 6.3 Procedure:

6.3.1 Place the sample from 9.2.2 or 17.1 in an appropriate anhydrous vial, and dissolve with approximately 0.5 mL of either methanol or toluene. Add approximately 1 mL of DMF-DMA, mix well, and maintain the sample at 30–40°C for 15 minutes.

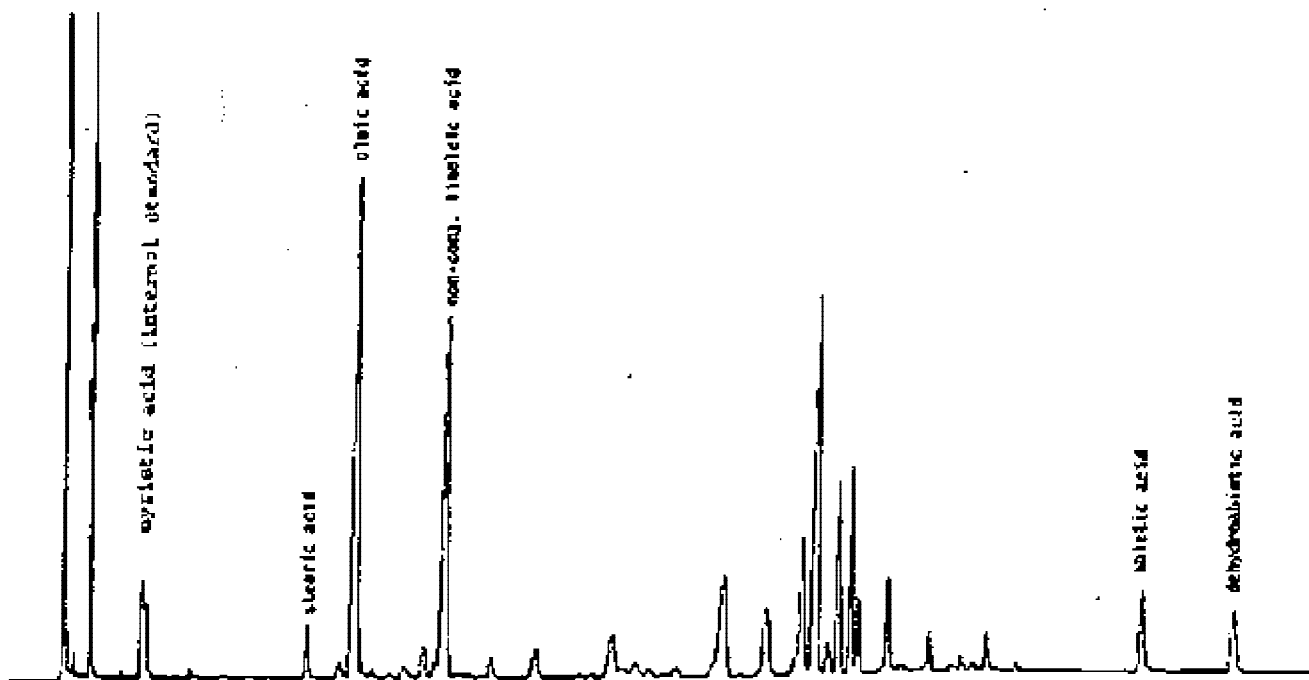
### INTERNAL STANDARD METHOD

#### 7. Apparatus

7.1 *Gas Chromatograph*— An instrument equipped with a flame ionization detector (FID) that can be operated at conditions given in 10.1.

7.2 *Column*—A high resolution column between 15 and 60 m in length, 0.25 to 0.53 mm internal diameter, with a 0.20- $\mu$ m film thickness of biscyanopropylsiloxane type liquid phase. The recommended referee column is 30 m in length, 0.32 mm internal diameter, with a 0.20- $\mu$ m film thickness, and provides separations equivalent or better than that displayed in Fig. 1.

<sup>4</sup> *Reagent Chemicals, American Chemical Society Specifications, American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary*, U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.*



NOTE 1—Labeled peaks are eluted as methyl esters.  
**FIG. 1 Typical Chromatogram of Distilled Tall Oil**

NOTE 4—When using this method for referee purposes, verify that the resolution is adequate and comparable to that shown in Fig. 1.

7.3 Analytical Balance, accurate to 0.1 mg.

**8. Reagents and Materials**

8.1 Purity of Reagents, see 4.2.1.

8.2 Myristic Acid (Internal Standard), 99 % pure.

NOTE 5—A higher molecular-weight saturated fatty acid that elutes as a methyl ester later in the chromatogram may be used in place of, or in addition to myristic acid, provided that the alternative internal standard peak does not coelute with sample component peaks.

8.3 Stearic Acid, Oleic Acid, Linoleic Acid, Abietic Acid, and Dehydroabietic Acid—Other high purity reference standards can be added as needed.

**9. Procedure**

9.1 Preparation of Calibration Standard :

9.1.1 Accurately weigh into a suitable vial, milligram quantities of the myristic acid internal standard, plus the fatty acid and rosin acid standards that are anticipated to be in the test sample, and record the weights.

9.1.2 Convert the calibration standard to the methyl esters or substituted ammonium salts as described in Sections 4, 5, 6, or 24.

9.2 Preparation of Test Sample:

9.2.1 Accurately weigh ~50 mg of sample and ~15 mg of myristic acid directly into a suitable vial and record the weight.

NOTE 6—Rosin samples need to be freshly broken from a larger mass to ensure the results are not affected by air oxidation of the rosin.

9.2.2 Convert the test sample to methyl esters or substituted ammonium salts, as described in Sections 4, 5, 6, or 24.

**10. Set-up of Gas Chromatograph (GC)**

10.1 Set the GC conditions so that they are approximately (see Note 7) as follows:

Column temperature (oven temperature)	
Initial	150°C
Hold	5 min
Ramp	5°C/min
Final	250°C
Hold	10 min
Injection port temperature	300°C
Injection port liner	glass split
Detector temperature	325°C
Carrier gas	helium
Linear gas velocity	19.5–20.5 cm/s
Split ratio	100 to 1 maximum
Detector	FID
Hydrogen	30 mL/min
Air	400 mL/min
Makeup gas	30 mL/min

NOTE 7—These are typical operating conditions only. The individual instrument should be adjusted in accordance with manufacturer’s instructions to optimize desired performance. Ongoing adjustments in operating temperature and flow rate may be necessary to maintain optimum performance of the column due to aging.

10.2 Calibration:

10.2.1 Inject 0.5 to 1.0 µL of the appropriate standard prepared in 9.1.

10.2.2 Record the retention time and calculate the individual relative response factors as follows:

**TABLE 1 Precision of Internal Standard Method**

	Repeatability Limit, 95 %	Repeatability Standard Deviation	Reproducibility Limit, 95 %	Reproducibility Standard Deviation
TOFA Material				
Stearic acid	0.6	0.2	0.8	0.3
Oleic acid	7.6	2.7	10.4	3.7
Linoleic acid	5.0	1.8	7.3	2.6
DTO Material				
Stearic acid	0.2	0.09	0.6	0.2
Oleic acid	1.4	0.5	1.7	0.6
Linoleic acid	1.1	0.4	1.4	0.5
Abietic acid	0.6	0.2	0.8	0.3
Dehydroabietic acid	0.3	0.1	0.8	0.3
ROSIN Material				
Abietic acid	6.4	2.3	10.6	3.8
Dehydroabietic acid	2.0	0.7	2.8	1.0

**TABLE 2 Precision of Area Percent Method**

	Repeatability Limit, 95 %	Repeatability Standard Deviation	Reproducibility Limit, 95 %	Reproducibility Standard Deviation
TOFA Material				
Stearic acid	0.3	0.1	0.6	0.2
Oleic acid	1.4	0.5	5.3	1.9
Linoleic acid	1.4	0.5	2.0	0.7
DTO Material				
Stearic acid	0.8	0.3	1.1	0.4
Oleic acid	0.6	0.2	2.8	1.0
Linoleic acid	0.8	0.3	1.4	0.5
Abietic acid	0.3	0.1	1.1	0.4
Dehydroabietic acid	0.6	0.2	1.4	0.5
ROSIN Material				
Abietic acid	1.4	0.5	4.2	1.5
Dehydroabietic acid	1.1	0.4	4.5	1.6

$$RRF_i = \frac{W_i}{A_i} \times \frac{A_{IS}}{W_{IS}} \quad (1)$$

where:

$RRF_i$  = relative response factor of individual fatty or rosin acid methyl esters,

$W_i$  = weight of individual fatty or rosin acid methyl esters in standard,  $W_i$  = weight used  $\times$  purity,

$A_i$  = peak area of individual fatty or rosin acid,

$A_{IS}$  = peak area of internal standard, and

$W_{IS}$  = weight of internal standard.  $W_{IS}$  = weight used  $\times$  purity.

NOTE 8—For highest accuracy, the purity of the standards should be used to correct the weight terms.

## 11. Analysis

11.1 Inject 0.5 to 1.0  $\mu$ L of the test sample prepared in 9.2.3.

NOTE 9—Dilution of the sample with additional solvent may be necessary to obtain injections that do not overload the column or detector.

## 12. Calculation

12.1 Obtain the peak areas of all of the peaks needed from the chromatogram.

NOTE 10—See Fig. 1 for chromatogram of a typical distilled tall oil (DTO).

12.2 Calculate the absolute value of each peak of interest, as follows:

$$\text{Fatty or rosin acid, \%} = \frac{A_i \times RRF_i \times W_{IS}}{A_{IS} \times W_s} \times 100 \quad (2)$$

where:

$A_i$  = peak area for fatty or rosin acid methyl ester being determined,

$RRF_i$  = relative response factor for individual compound being determined,

$W_{IS}$  = weight of internal standard.  $W_{IS}$  = weight used times purity,

$A_{IS}$  = peak area of internal standard, and

$W_s$  = sample weight.

## 13. Report

13.1 Report the percentage of the individual fatty and rosin acids to the nearest 0.1 %.

## 14. Precision and Bias

14.1 *Internal Standard Method*—An interlaboratory study of the capillary GC determination of fatty and rosin acids in tall oil fatty acids (TOFA), DTO, and rosin was run in 1995 by nine laboratories. The design of the experiment, similar to that of Practice E 691, and a within-between analysis of the data are given in ASTM Research Report.<sup>5</sup>

14.1.1 *Test Result*—The precision information given in Table 1 for fatty and rosin acids is for the comparison of two test results.

NOTE 11—Repeatability = within laboratory, Reproducibility = between laboratories.

<sup>5</sup> Supporting data are available from ASTM Headquarters. Request RR:D01-1101.

14.1.2 *Bias*—Since there is no accepted reference material, method or laboratory suitable for determining the bias for the procedure in this test method for measuring component concentration, no statement on bias is being made.

### AREA PERCENT METHOD

**15. Apparatus**—Same as apparatus described in Section 7.

### 16. Reagents and Materials

16.1 *Purity of Reagents*—See 4.2.1.

### 17. Procedure

17.1 *Preparation of Test Sample*—Weigh approximately 50 mg of sample into a suitable vial, and convert to methyl esters or substituted ammonium salts as described in Sections 4, 5, 6, or 24.

NOTE 12—Rosin samples need to be freshly broken from a larger mass to ensure the results are not affected by air oxidation of the rosin.

### 18. Set-up of Gas Chromatograph

18.1 Set the GC conditions as described in 10.1.

### 19. Analysis

19.1 Inject 1  $\mu\text{L}$  of the test sample prepared in 17.1.

### 20. Calculation

20.1 Sum all the areas of the individual peaks, exclusive of the solvent peak, to obtain the total peak area.

20.2 Calculate the relative percent of each fatty and rosin acid methyl ester present, uncorrected for the amount of polymeric materials present, as follows:

$$\text{Fatty or rosin acid, \%} = (A \times 100)/TA \quad (3)$$

where:

A = peak area for fatty or rosin acid methyl ester being determined, and

TA = sum of areas of all fatty acid and rosin acid methyl ester peaks.

NOTE 13—See Fig. 1 for chromatogram of a typical DTO.

### 21. Report

21.1 Report the area percent of the individual fatty and rosin acids to the nearest 0.1 %.

### 22. Precision and Bias

22.1 *Area Percent Method*—An interlaboratory study of the capillary GC determination of fatty and rosin acids in TOFA, DTO, and rosin was run in 1995 by nine laboratories. The design of the experiment, similar to that of Practice E 691, and a within-between analysis of the data are given in an ASTM Research Report.<sup>5</sup>

22.1.1 *Test Result*—The precision information given in Table 2 for fatty and rosin acids concentration of three naval stores products, is for the comparison of two test results.

22.1.2 *Bias*—Since there is no accepted reference material, method or laboratory suitable for determining the bias for the procedure in this test method for measuring component concentration, no statement on bias is being made.

### 23. Keywords

23.1 area percent; derivatization; fatty acids; gas chromatography; internal standard; rosin acids

## APPENDIX

### (Nonmandatory Information)

#### X1. CONVERSION BY MEANS OF DIAZOMETHANE

##### X1.1 Apparatus:

X1.1.1 *Test Tube*, four, 25 by 200 mm.

X1.1.2 *Standard Laboratory Glassware*.

##### X1.2 Reagents and Materials:

X1.2.1 *Purity of Reagents*—see 4.2.1.

X1.2.2 *N-Methyl-N-Nitroso-p-Toluenesulfonamide*, CAS No. 80-11-5.

X1.2.3 *Diethyl Ether*, anhydrous.

X1.2.4 *2-Ethoxyethanol*.

X1.2.5 *Methanol*, anhydrous.

X1.2.6 *Potassium Hydroxide Solution*, aqueous, 40 % weight/volume (w/v).

X1.2.7 *Toluene*.

##### X1.3 Procedure :

X1.3.1 **Warning**—Diazomethane is explosive and an insidious poison. Use extreme care in using the following esterification procedure. The procedure must be performed in a fume hood.

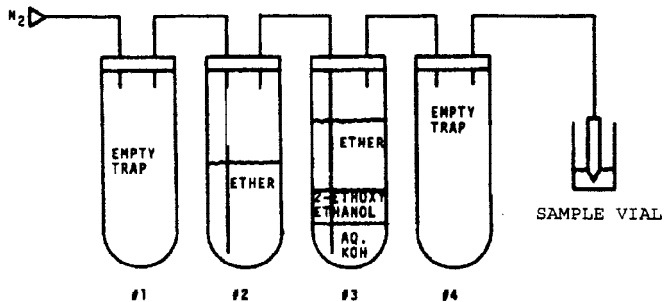
X1.3.2 Dissolve the sample from 9.2.2 or 17.1 in 0.5 mL of 10 % (v/v) methanol/diethyl ether.

NOTE X1.1—For solid rosin, or other samples that are difficult to dissolve, 2 to 3 drops of toluene may be added to the vial prior to derivatization, to assist in dissolving the sample.

X1.3.3 Connect four 25 by 200-mm test tubes as shown in Fig. X1.1. Use rubber stoppers in Tubes 1 and 4 and cork stoppers in Tubes 2 and 3. Connect the tubes with glass tubing as shown. Draw down the outlet end of the glass tube from Test Tube 4 to about a 1 to 2-mm diameter opening.

X1.3.4 Leave Test Tubes 1 and 4 empty, to serve as traps. Fill Tube 2 about one half full with diethyl ether.





NOTE—Connect four 25 by 200-mm test tubes in the hood as shown. Use rubber stoppers on Test Tubes 1 and 4; use cork stoppers on Test Tubes 2 and 3.

NOTE 1—Connect four 25 by 200-mm test tubes in the hood as shown. Use rubber stoppers on Test Tubes 1 and 4; use cork stoppers on Test Tubes 2 and 3.

FIG. X1.1 Apparatus for Diazomethane Methylation

X1.3.5 Add about 10 mL of the KOH solution, 10 mL of 2-ethoxyethanol, and 20 mL of diethyl ether to Test Tube 3.

X1.3.6 Place the vial with the methanol/ether solution of the sample under the delivery tube from Test Tube 4, so that the end of the tube dips beneath the surface of the liquid.

X1.3.7 Add about 1 g of *N-methyl-N-nitroso-p-toluenesulfonamide* to Test Tube 3. Apply nitrogen gas to the inlet of Test Tube 1 at such a rate as to sweep the generated diazomethane through the solution of the sample at a moderate rate.

X1.3.8 The esterification is complete when the yellow color of excess diazomethane is apparent in the sample vial.

X1.3.9 Evaporate the solution almost to dryness under a very gentle stream of nitrogen on a steam bath in a hood. Dilute with ether or other suitable solvent to about 0.5 to 0.6 mL.

NOTE X1.2—An alternative procedure would be to use a heating block at 50°C to evaporate the solvent. Regardless of the method, care must be taken not to lose the more volatile esters.

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