Designation: E2997 – 16

Standard Test Method for Analysis of Biodiesel Products by Gas Chromatography-Mass Spectrometry[1]

This standard is issued under the fixed designation E2997; 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 analysis and identification of the fatty acid methyl esters (FAMEs) and petroleum distillate components of biodiesel products.

1.2 This test method is suitable for identifying the components of biodiesel products in extracts of debris samples or in liquid samples.

1.3 The identification of a specific source of the FAMEs or the proportion of the blend of biodiesel requires additional analysis and is beyond the scope of this test method.

1.4 This test method cannot replace the requisite knowledge, skills, or abilities acquired through appropriate education, training, and experience and should be used in conjunction with sound professional judgment.

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

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

2. Referenced Documents

2.1 *ASTM Standards:*[2]

E620 Practice for Reporting Opinions of Scientific or Technical Experts

E1386 Practice for Separation of Ignitable Liquid Residues from Fire Debris Samples by Solvent Extraction

E1412 Practice for Separation of Ignitable Liquid Residues from Fire Debris Samples by Passive Headspace Concentration With Activated Charcoal

E1413 Practice for Separation of Ignitable Liquid Residues from Fire Debris Samples by Dynamic Headspace Concentration

E1492 Practice for Receiving, Documenting, Storing, and Retrieving Evidence in a Forensic Science Laboratory

E1618 Test Method for Ignitable Liquid Residues in Extracts from Fire Debris Samples by Gas Chromatography-Mass Spectrometry

E2154 Practice for Separation and Concentration of Ignitable Liquid Residues from Fire Debris Samples by Passive Headspace Concentration with Solid Phase Microextraction (SPME)

E2451 Practice for Preserving Ignitable Liquids and Ignitable Liquid Residue Extracts from Fire Debris Samples

E2881 Test Method for Extraction and Derivatization of Vegetable Oils and Fats from Fire Debris and Liquid Samples with Analysis by Gas Chromatography-Mass Spectrometry

3. Summary of Test Method

3.1 Traditional ignitable liquid analysis will be used to identify biodiesel products.

3.2 The debris sample is extracted or an aliquot of a liquid is extracted or diluted and analyzed by gas chromatography-mass spectrometry (GC-MS).

3.3 If fatty acid methyl esters (FAMEs) are suspected, further solvent extraction and analysis on a FAME-specific column may be required.

3.4 Specific chemical components (fatty acid methyl esters) are identified by their retention times and mass spectra.

4. Significance and Use

4.1 This test method specifically identifies fatty acid methyl esters and petroleum distillates found in biodiesel products. Derivatization is not necessary to identify FAMEs.

4.1.1 This test method is useful when biodiesel products are suspected as a fuel source in a fire or a fuel product case and the identification of the "bio" portion of the fuel is of interest.

4.1.2 The identification of biodiesel in samples from a fire scene can support the field investigator's opinion regarding the origin and cause of the fire or provide investigative leads.

[1] This test method is under the jurisdiction of ASTM Committee E30 on Forensic Sciences and is the direct responsibility of Subcommittee E30.01 on Criminalistics.

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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.

4.1.3 The identification of biodiesel in a sample of fuel from a dispensing container or fuel tank can support the field investigator's findings in a fuel product tampering investigation.

4.2 FAMEs can be identified in debris samples using headspace extraction techniques and GC-MS.

4.2.1 Solvent extraction may be required to further identify and characterize the FAME content in biodiesel.

4.2.2 The presence of FAMEs and petroleum diesel is a strong indication of a biodiesel product, as FAMEs are not naturally occurring.

4.3 Biodiesel products can be identified in liquid samples by GC-MS after appropriate solvent dilution or extraction.

4.4 Biodiesel is available in different blends, where B100 is 100 % biodiesel (typically 100 % transesterified vegetable oils or recycled restaurant greases) and B2 is 2 % biodiesel and 98 % petroleum diesel, with variations in between.

4.5 This test method is a sensitive technique and can detect quantities as small as 7 µL of biodiesel residue in an extract from a debris sample.

4.6 This test method can be hampered by coincident extraction of interfering compounds present in the debris samples.

4.7 Depending on the extraction method used, this could be a destructive technique and whenever possible the entire sample should not be used for the procedure. Solvent extracted portions of the sample are not suitable for resampling.

4.8 Alternate methods of extraction or analysis exist and may be suitable for use in obtaining similar results and conclusions.

5. Apparatus

5.1 *Gas Chromatograph*—A chromatograph capable of using capillary columns and being interfaced to a mass spectrometer.

5.1.1 *Sample Inlet System*—A sample inlet system that can be operated in either split or splitless mode with capillary columns; the inlet system may use on-column technology.

5.1.2 *Column*—A non-polar capillary, bonded phase, methylsilicone or phenylmethylsilicone column or equivalent, or a polar capillary, bonded phase column, such as a cyanopropyl-based fatty acid specific column or equivalent, may be used to determine the presence of fatty acids.

5.1.2.1 A polar capillary, bonded phase column, such as a cyanopropyl-based fatty acid specific column or equivalent shall be used to perform comparative analysis between specimens of questioned neat liquid samples, debris extracts, or both.

5.1.2.2 Any column length or temperature program conditions may be used for the polar column provided that each component of the reference mixture (see 6.6.2) is adequately separated.

5.1.3 *GC Oven*—A column oven capable of reproducible temperature program operation in the range from 50 to 300°C.

5.2 *Mass Spectrometer*—Capable of acquiring mass spectra from m/z 40 to m/z 400 with unit resolution or better, with continuous data output.

5.2.1 *Sensitivity and Resolution*—The system shall be capable of detecting each component of the reference or test mixture (see 6.6) and providing sufficient ion intensity data to identify each component, either by computer search of a mass spectral library or by comparison with reference spectra.

5.3 *Data Station*—A computerized data station capable of storing time sequenced mass spectral data from sample runs.

5.3.1 *Data Handling*—The data system shall be capable of performing, either through its operating system or by user programming, various data handling functions, including input and storage of sample data files, generation of extracted ion profiles, searching data files for selected compounds, and qualitative and semi-quantitative compound analysis.

5.3.2 *Mass Spectral Libraries*—The system shall be capable of retrieving a specified mass spectrum from a data file and comparing it against a library of mass spectra available to the data system. This capability is considered an aid to the analyst, who will use it in conjunction with chromatographic data and known reference materials to identify unknown components.

5.4 *Syringes*—A syringe capable of introducing a sample size in the range from 0.1 to 10.0 µL.

5.5 *Steam bath or heating device,* for use in warming sample extracts in containers used during evaporation steps.

6. Reagents and Materials

6.1 *Purity of Reagents*—Reagent grade chemicals should be used in all tests. It is intended that all reagents conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society.[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.

6.2 *Solvent*—A suitable solvent, such as n-pentane, carbon disulfide, or other solvent that will not interfere with the analysis.

6.2.1 Solvent purity can be verified by evaporating to at least twice the extent used in the analysis and analyzing the evaporated solvent in accordance with Test Method E1618.

6.3 *Filter apparatus,* free of extractable hydrocarbons, oils, and fats.

6.4 *Evaporation accelerants,* compressed dry nitrogen, filtered air, or inert gas used in the concentration of solvent extracts.

6.5 *Carrier gas,* helium or hydrogen of purity 99.995 % or higher.

6.6 *Reference and Test Mixtures:*

6.6.1 Refer to Test Method E1618, Section 6, for the appropriate test mixture for the hydrocarbon portion of the analysis.

6.6.2 Refer to Test Method E2881, Section 6, for the appropriate reference mixture for the "bio" portion of the analysis.

[3] *Reagent Chemicals, American Chemical Society Specifications*, American Chemical Society, Washington, DC.

6.6.3 Reference biodiesel products should be obtained for comparison and identification purposes. Typical chromatograms of B100 and B20 on a non-polar fire debris column are shown in Fig. 1. Typical chromatograms of B100 and B20 on a fatty acid specific polar column are shown in Fig. 2.

6.7 *Glassware or labware,* clean glassware (beakers, test tubes, and vials) or disposable labware free of extractable hydrocarbons, oils, and waxes.

7. Equipment Calibration and Maintenance

7.1 Verify the consistent performance of the chromatographic instrument by using blanks and a known concentration of the appropriate reference or test mixture (see 6.6). Optimize gas flow periodically.

7.2 Tune and check calibration of mass spectrometer.

7.2.1 Ensure proper operation of the mass spectrometer using perfluorotributylamine (PFTBA), or another appropriate calibration standard, according to the instrument manufacturer's specifications, prior to use. This should be done at least every day that the instrument is used or in accordance with manufacturer's recommendations.

7.2.2 Maintain tuning documentation as a portion of the quality control documentation.

7.3 *Equipment Maintenance:*

7.3.1 Change septa, replace gold seals, trim front end of column, and clean or replace injector liners on a periodic basis to avoid sample contamination by carryover of residual material from previous sample injections.

8. Sample Handling/Analysis Procedure

8.1 Observe the appropriate procedures for handling and documentation of all submitted samples as described in Practice E1492.

8.1.1 Open and examine the fire debris or liquid sample in order to determine that it is consistent with its description. Resolve any discrepancies between the submitter's description of the sample and the analyst's observations prior to the completion of the report.

8.2 Only specimens of appropriate dilution should be analyzed on a GC-MS system.

8.3 Analyze solvent blanks at least once each day that the instrument is used; maintain these analysis records as a portion of the quality control documentation. This will verify the purity of the solvent and potentially detect carryover or contamination.

8.4 Clean syringes thoroughly between injections to ensure no carryover.

8.4.1 Conduct carryover studies, and maintain records that demonstrate the adequacy of laboratory procedures to prevent carryover.

8.4.2 Running solvent blanks between each specimen is recommended but not required if studies demonstrate that the cleaning procedure is adequate to prevent carryover.

8.5 Maintain reference files of known biodiesel products that have been prepared and analyzed in the same manner as the questioned samples.

8.6 Extract a fire debris sample using one or more techniques as described in Practices E1386, E1412, E1413, or E2154 to isolate ignitable liquid residues and FAME components for analysis.

8.7 **Warning—**Extract a fire debris sample using headspace techniques prior to performing any solvent extraction if the identification of all classes of ignitable liquids is of interest.

8.8 An aliquot of a liquid sample is extracted or diluted as appropriate.

8.9 Analyze the sample specimen(s) along with reference materials or standards, as necessary, to identify the components of biodiesel products and other ignitable liquid residues in accordance with Test Method E1618 (also see Fig. 1).

8.9.1 If any FAMEs are detected, additional analysis may be required to further identify the FAME components. Practice E1386 may be followed to extract a portion of the original sample, or the original extract can be concentrated, followed by analysis on a FAME-specific column using conditions as suggested in Fig. 2 and 5.1.2.2.

8.9.2 Extracts may be further concentrated for re-analysis. Place the extract in a chemical fume hood and evaporate at room temperature to a suitable final volume. Compressed dry nitrogen, filtered air, or an inert gas can be used to accelerate evaporation. Using heat to facilitate concentration is acceptable for suspected biodiesel samples as polymerization does not occur with FAMEs.

8.9.2.1 **Warning—**Do not evaporate an extract to dryness if lighter ignitable liquid residues were identified and no other sample extract is available for archiving and long-term storage.

8.10 See Annex A1 for sample and extract storage guidance.

9. Data Analysis and Interpretation

9.1 The identification of FAMEs in liquids and fire debris extracts is performed using retention time and mass spectral data compared with certified standards or reference materials (see 6.6). The presence of FAMEs, with or without petroleum diesel, is an indication of biodiesel, as FAMEs are not naturally occurring. Refer to Test Method E1618 for identification of petroleum distillates. Typical chromatograms of B100 and B20 on a traditional non-polar fire debris column collected following Practice E1412 are shown in Fig. 3.

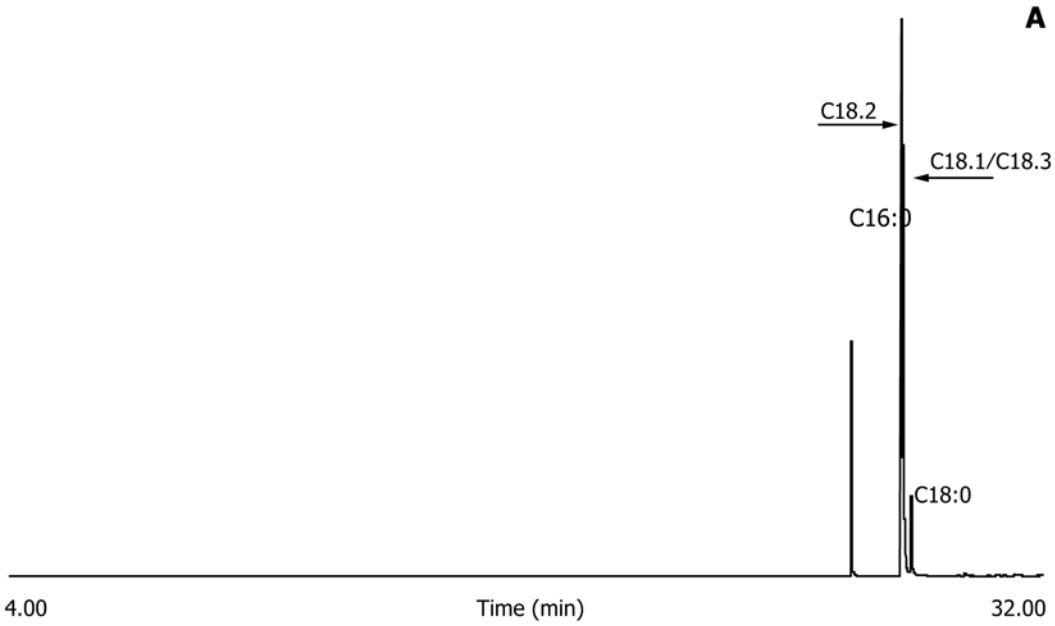
9.1.1 **Warning—**Some household and commercial products, such as some laundry detergents and fabric softeners, may contain FAMEs. Debris samples should be thoroughly inspected after analysis for items or materials that could be a source of FAMEs in an extract other than a biodiesel fuel.

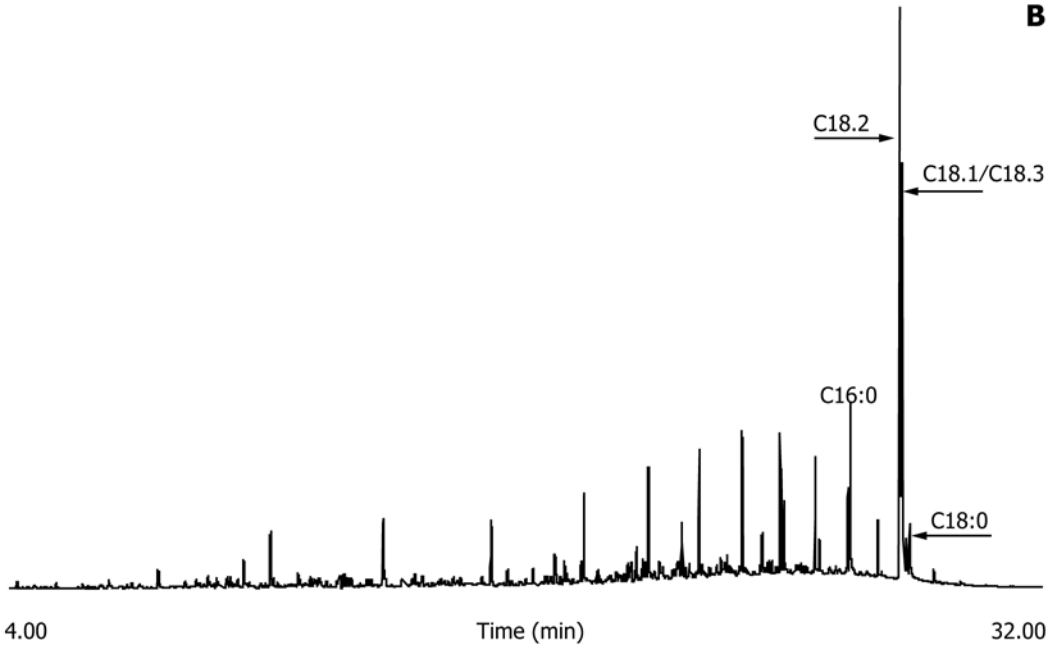
9.2 *Extracted ion Profiling (EIP):*

9.2.1 Extracted ion profiles may be used to elucidate petroleum distillates and fatty acid methyl ester peaks from other interferences in the chromatograms.

9.2.2 A data station is used to extract and draw extracted ion profiles (mass chromatograms) for major ions characteristic of petroleum distillates and fatty acid methyl esters.

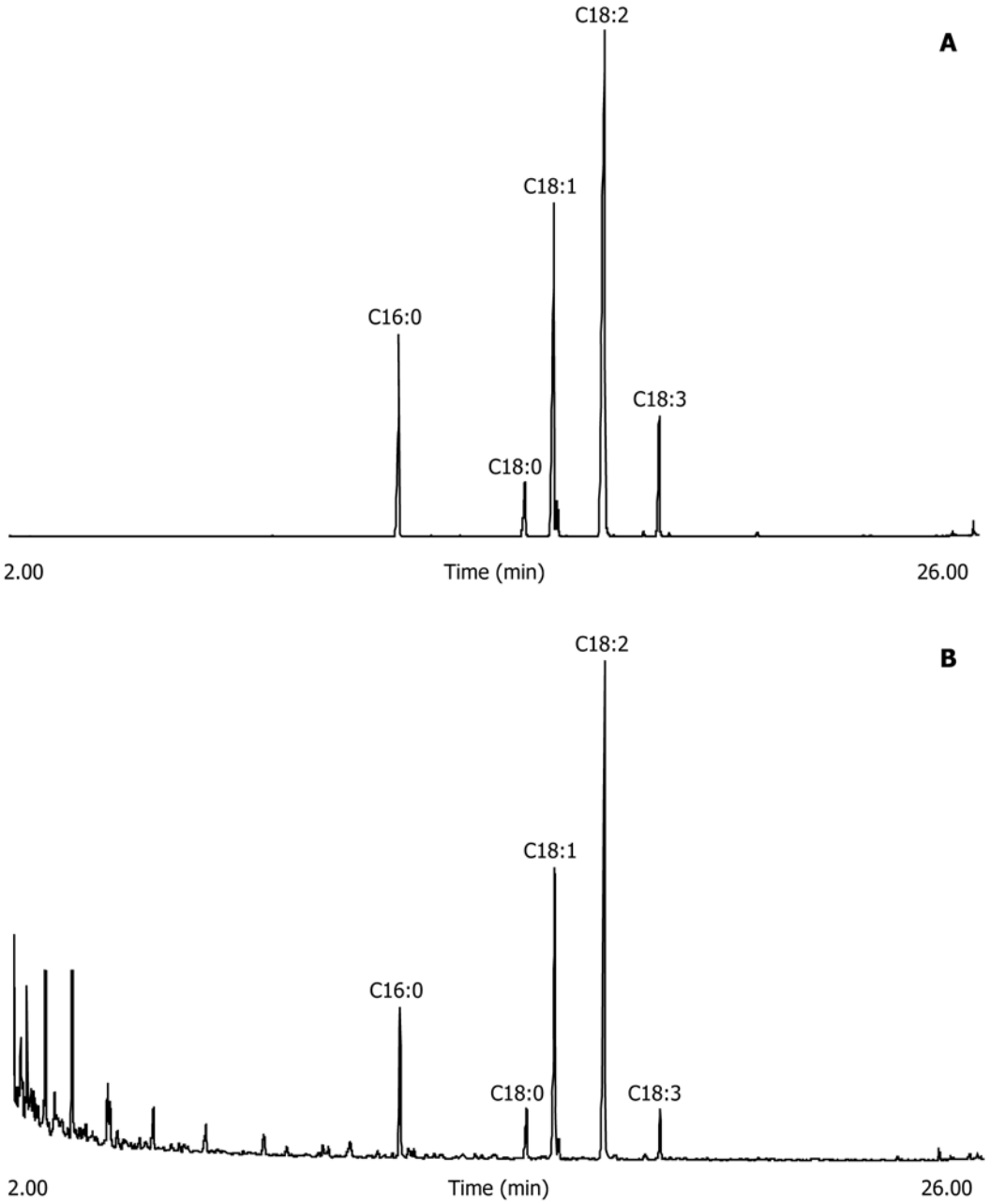
9.2.3 Typically ions 67, 74, and 79 m/z or the molecular ion for the fatty acid methyl ester of interest can be summed together to form a useful EIP. Refer to Table 1 in Test Method E2881 for a list of common fatty acids and the molecular





Column:	30 m x 0.25 mm x 0.25 µm, DB-1MS (polydimethylsiloxane) or equivalent
Mobile Phase:	Helium, 1.2 mL/min
Sample:	1 µL
Injector:	250°C
Initial Temperature:	40°C for 2 min
Rate:	5°C/min to 120°C for 0 min
	12°C/min to 300°C for 5 min
Total Run Time:	38 min
Split:	20:1

FIG. 1 Total Ion Chromatogram (TIC) of B100 (A) and B20 (B) Reference Material on a DB-1MS Capillary Column Using Test Method E1618 GC-MS Conditions



Column:	30 m x 0.25 mm x 0.20 µm, SP-2380 poly(90% biscyanopropyl/ 10% cyanopropylphenyl siloxane) or equivalent
Mobile Phase:	Helium, 1.0 mL/min
Sample:	1 µL
Injector:	250°C
Initial Temperature:	105°C for 0 min
Rate:	4°C/min to 200°C for 0 min 20°C/min to 260°C for 0 min
Total Run Time:	26.75 min
Split:	20:1

FIG. 2 Total Ion Chromatogram (TIC) of B100 (A) and B20 (B) Reference Material on a SP-2380 Capillary Column Using a Program Optimized for Oil and Fat Analysis

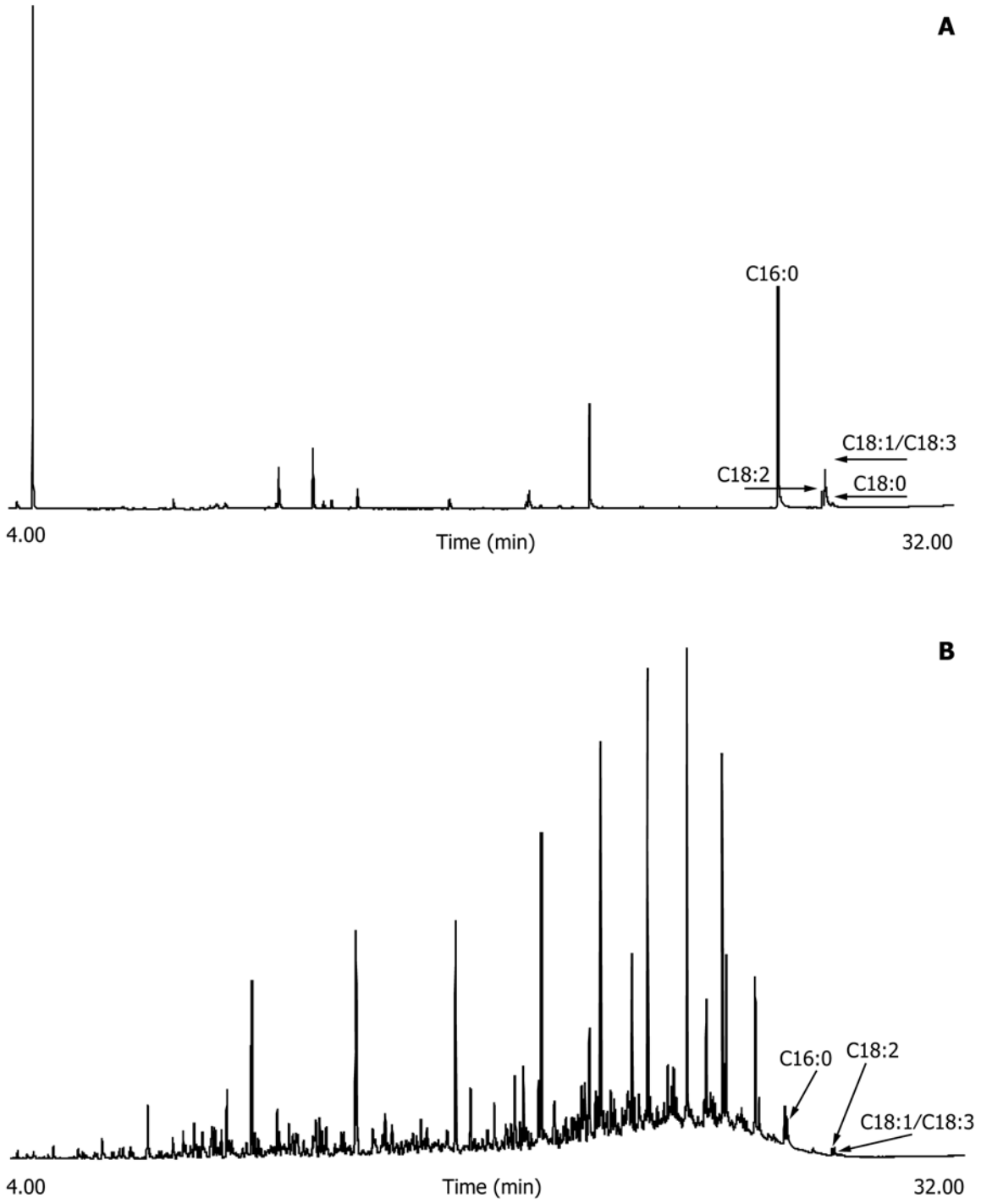


FIG. 3 Total Ion Chromatogram (TIC) of B100 (A) and B20 (B) Reference Material Following GC-MS Conditions Listed in Fig. 1; Chromatograms Represent 10 µL of Each Reference Material Spiked onto a Lab Wipe in a Metal Quart Can; One Half of Charcoal Strip was Suspended in Each Can and Extracted Approximately 16 Hours at 65°C; the Charcoal Strip was Eluted Using Approximately 400 µL Carbon Disulfide

weights of the resulting methyl esters. Typical EIPs of B100 and B20 on a traditional non-polar fire debris column collected following Practice E1412 are shown in Fig. 4.

9.2.4 Refer to Test Method E1618 for general information on extracted ion profiles and chromatographic characteristics of petroleum distillates.

9.3 A comparative analysis between specimens from questioned neat liquid samples, debris extracts, or both shall be conducted with data from specimens analyzed on a polar capillary, bonded phase column, such as a cyanopropyl-based fatty acid specific column or equivalent.

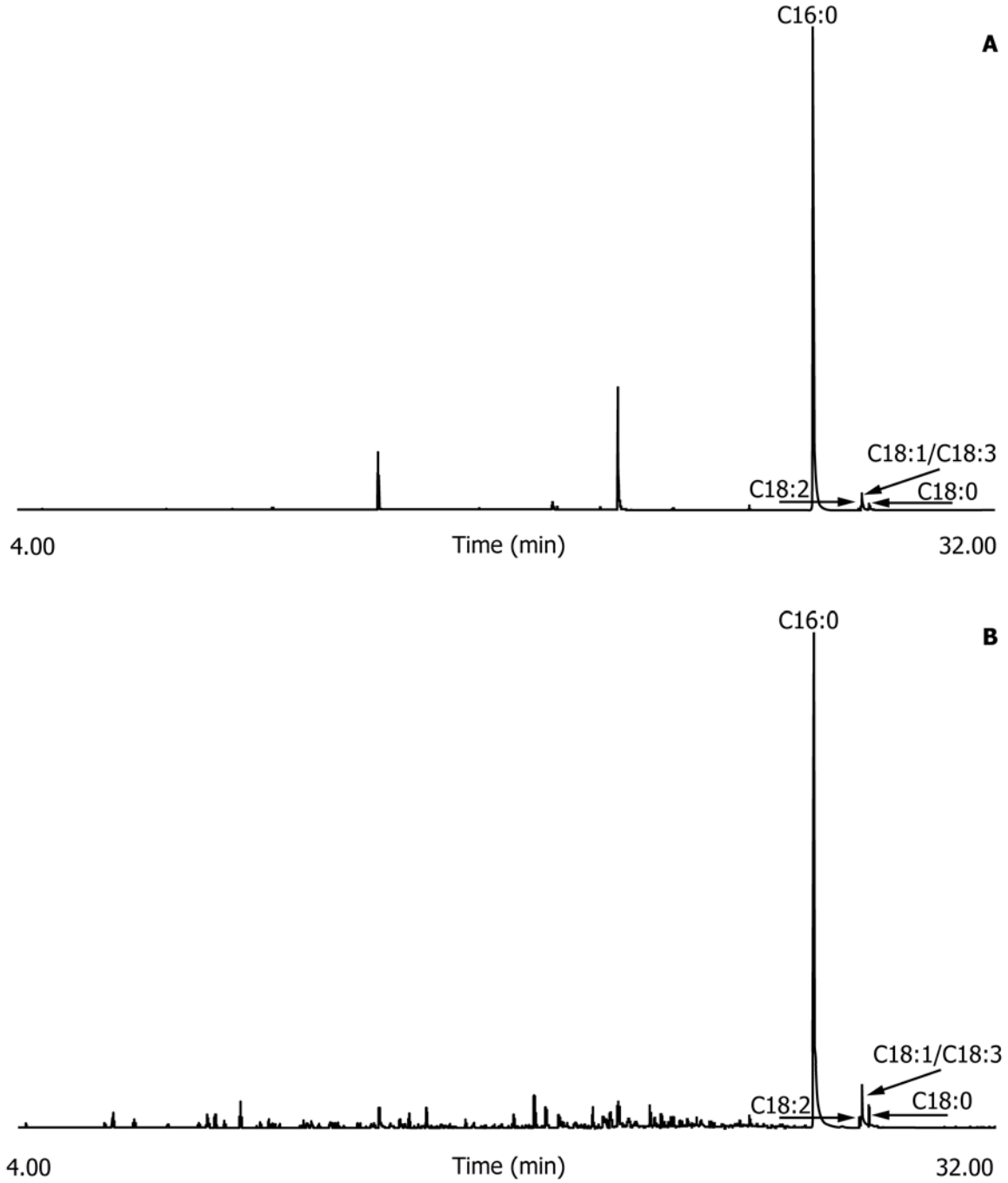


FIG. 4 Extracted Ion 74 m/z of B100 (A) and B20 (B) Reference Materials in Fig. 3

9.3.1 Care should be taken when comparing liquid to debris samples, as patterns are markedly different depending on the sample preparation technique and the effect of fire.

10. Report Wording

10.1 *Suggested Wording for Liquid and Debris Samples Containing FAMEs:*

10.1.1 Fatty acid methyl esters, which are indicative of a biodiesel product, were identified. Biodiesel products are ignitable liquids.

10.1.2 A heavy petroleum distillate (HPD) and fatty acid methyl esters were identified. This combination is indicative of a biodiesel product. Biodiesel products are ignitable liquids.

10.1.2.1 Biodiesel products except for B100 can be classified using the Ignitable Liquid Classification Scheme (see Table 1 in Test Method E1618) as a "Heavy Petroleum Distillate" containing FAMEs or as an "Others-Miscellaneous" blended product. B100 should be classified as an "Oxygenated" product comprised of FAMEs.

10.1.3 *Suggested Wording in Fuel Tampering Cases:*

10.1.3.1 Sample #1 (liquid from vehicle fuel tank) and Sample #2 (Company Y biodiesel fuel) contained a heavy petroleum distillate (HPD) and fatty acid methyl esters. Sample #3 (liquid from Company Y fuel delivery truck) contained only an HPD.

10.2 *Suggested Wording for Negative Reports for Fire Debris Cases:*

10.2.1 No ignitable liquids were identified. The absence of an ignitable liquid residue does not preclude the possibility that ignitable liquids were present at the fire scene. Ignitable liquids are volatile compounds that may have evaporated, been totally consumed in a fire, environmentally altered or removed, or otherwise indistinguishable from background materials.

10.3 Refer to Practice E620 and Test Method E1618 for general information on report writing.

11. Limitations

11.1 The HPD portion of a biodiesel specimen must be identified using a non-polar capillary bonded phase column as specified in 5.1.2 (and Test Method E1618).

11.1.1 The FAME portion of a biodiesel specimen may be identified by either a non-polar capillary column or a polar capillary, bonded phase column as specified in 5.1.2 (and Test Method E1618).

11.2 This is a qualitative technique. The identification of a specific source of the FAMEs or the proportion of the blend of biodiesel requires additional analysis and is beyond the scope of this test method.

12. Precision and Bias

12.1 This test method is qualitative, and therefore measures of precision and bias are not applicable.

13. Keywords

13.1 biodiesel; fatty acid methyl esters; fire debris samples; forensic sciences; gas chromatography; ignitable liquid residues; mass spectrometry; vegetable oils

ANNEX

(Mandatory Information)

A1. SAMPLE STORAGE

A1.1 After extraction, store the original debris or liquid sample using appropriate procedures for handling and documentation as set forth in Practice E1492.

A1.2 *Extract Storage Short-Term*—Extracts, liquid samples, or solvents may be stored in septum seal or screw cap glass vials with PTFE-lined seals to prevent evaporation.

A1.3 *Extract Storage Long-Term*—Follow the procedures set forth in Practice E2451 for long-term storage of extracts or liquid samples.

RELATED MATERIAL

Kuk, R. J., Spagnola, M. V., "Extraction of Alternative Fuels from Fire Debris Samples," *Journal of Forensic Sciences*, Vol 53, No. 5, September 2008, pp. 1123–1129.

Stauffer, E., Byron, D., "Alternative Fuels in Fire Debris Analysis: Biodiesel Basics," *Journal of Forensic Sciences*, Vol 52, No. 2, March 2007, pp. 371–379.