



Standard Test Method for Extraction and Derivatization of Vegetable Oils and Fats from Fire Debris and Liquid Samples with Analysis by Gas Chromatography-Mass Spectrometry¹

This standard is issued under the fixed designation E2881; 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 extraction, derivatization, and identification of fatty acids indicative of vegetable oils and fats in fire debris and liquid samples. This procedure will also extract animal oils and fats, as these are similar in chemical composition to vegetable oils and fats. Herein, the phrase “oils and fats” will be used to refer to both animal and vegetable derived oils and fats.

1.2 This test method is suitable for successfully extracting oil and fat residues having 8 to 24 carbon atoms.

1.3 The identification of a specific type of oil (for example, olive, corn, linseed) requires a quantitative analysis of the fatty acid esters 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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

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.*

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. 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

E1388 Practice for Static Headspace Sampling of Vapors from Fire Debris Samples

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 onto an Adsorbent Tube

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

3. Summary of Test Method

3.1 If ignitable liquid analysis is required, it shall be performed prior to analysis for oils and fats as this test method is a destructive technique. A fire debris sample (or sub-sample) or an aliquot of a liquid is initially analyzed for ignitable liquid residues using standards listed in the referenced documents.

3.2 The same sample of fire debris (or different sub-sample) or an additional aliquot of a liquid is then extracted with an organic solvent, and a derivatizing agent is added to convert

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.

either the free fatty acids and some triglycerides (for acid-catalyzed derivatization) or just the triglycerides (for base-catalyzed derivatization) to fatty acid methyl esters (FAMES).

3.3 The organic layer of solvent is removed, filtered, and concentrated if necessary, using dry nitrogen, filtered air, or inert gas.

3.4 The derivatized extract is analyzed by gas chromatography-mass spectrometry (GC-MS).

3.5 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 is useful when oils and fats are suspected as an ignition source or a fuel source in a fire.

4.1.1 The identification of oil and fat residues in samples from a fire scene can support the field investigator's opinion regarding the origin and cause of the fire.

4.1.2 The positive identification of fatty acid(s) does not necessarily mean that the fire was caused by self heating.

4.2 This test method specifically identifies fatty acid derivatives. Oils and fats are comprised primarily of triglycerides (which are fatty acids attached to a glycerol backbone), and some free fatty acids. Free fatty acids and triglycerides are not easily analyzed by the traditional ignitable liquid extraction techniques. Solvent extraction and derivatization to FAME will enable identification by GC-MS.

4.2.1 The identification of an individual fatty acid in fire debris samples does not confirm the presence of oils or fats; however, there are times when large quantities of the oil or fat may be extracted. In such cases a more positive identification can be made.

4.2.2 Oils and fats containing fatty acids with no double bonds will generally have no tendency to self-heat. With increasing unsaturation (1, 2, and 3 double bonds), the tendency to self-heat also increases, such that polyunsaturated fatty acids (PUFAs), such as C18:3, have a high tendency to self-heat.

4.3 This test method is a sensitive separation technique and can detect quantities as small as 3 μ L of oil or fat residue in an extract from a debris sample.

4.4 This test method shall be performed after all required traditional testing for ignitable liquid residues is completed.

4.5 This test method extracts liquids and residues from porous and nonporous materials of various sizes.

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

4.7 This is a destructive technique and whenever possible the entire sample should not be used for the procedure. It is recommended that visual inspection be used to locate portions or areas exhibiting potential oily residue for sub-sampling which would preserve remaining portions for further analyses and also minimize solvent waste. The solvent extracted portions of the sample are not suitable for resampling.

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

4.9 Biodiesel, an ignitable liquid, is a trans-esterified product containing FAMES. The FAME compounds in biodiesel can be detected in fire debris using many fire debris extraction techniques followed directly by GC-MS analysis. Derivatization is not necessary to identify the FAMES in biodiesel.

4.10 For more information on oils, FAME, and fire debris analysis, see the references listed.^{3, 4, 5, 6}

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

5.1.2.1 A polar capillary, bonded phase, such as a cyanopropyl-based fatty acid specific column shall be used to perform comparative analysis between neat liquid samples, or fire debris samples, or both. Any column length or temperature program conditions may be used provided that each component of the reference mixture (see 6.8) is adequately separated on the polar column.

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 mixture (see 6.8) and providing sufficient ion intensity data to identify each component, either by computer library search 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

³ Gambrel, A. K., and Reardon, M. R., "Extraction, Derivatization, and Analysis of Vegetable Oils from Fire Debris," *Journal of Forensic Sciences*, Vol 53, No. 6, 2008, pp. 1372–1380.

⁴ Schwenk, L. M., and Reardon, M. R., "Practical Aspects of Analyzing Vegetable Oils in Fire Debris," *Journal of Forensic Sciences*, Vol 54, No. 4, 2009, pp. 874–880.

⁵ Stauffer, E., "A Review of the Analysis of Vegetable Oil Residues from Fire Debris Samples: Spontaneous Ignition, Vegetable Oils, and the Forensic Approach," *Journal of Forensic Sciences*, Vol 50, No. 5, 2005, pp. 1091–1100.

⁶ Stauffer, E., "A Review of the Analysis of Vegetable Oil Residues from Fire Debris Samples: Analytical Scheme, Interpretation of the Results, and Future Needs," *Journal of Forensic Sciences*, Vol 51, No. 5, 2006, pp. 1–17.

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, for use in warming sample extracts in containers used during evaporation steps.

6. Reagents and Materials

6.1 Purity of Reagents—Reagent grade chemicals shall 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. 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, n-hexane, or n-heptane.

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 Derivatization Reagent—There are two types of derivatization processes: (1) acid-catalyzed, which will act on both triglycerides and free fatty acids; and (2) base-catalyzed, which will only trans-esterify triglycerides. A suitable derivatization reagent, such as a 2N potassium hydroxide (KOH) in methanol solution (base-catalyzed) or a 10 % boron trifluoride in methanol solution (acid-catalyzed), will be chosen to convert the fatty acids and triglycerides to FAMES.

6.4 Drying Agent—A suitable drying agent, such as anhydrous sodium sulfate.

6.5 Filter Apparatus, free of extractable hydrocarbons, oils, and fats.

6.6 Evaporation Accelerants—Compressed dry nitrogen, filtered air, or inert gas used in the concentration of solvent extracts.

6.7 Carrier Gas—Helium or hydrogen of purity 99.995 % or higher.

6.8 Reference Mixture—The reference mixture shall consist of a minimum of the following FAMES: C16:0, C18:0, C18:1, C18:2, C18:3. Additional compounds may be included at the discretion of the analyst. The mixture should contain approximately equal parts by weight of the required fatty acids methyl esters in the chosen solvent or a traceable commercially available reference mixture. The final solution is prepared by diluting the above mixture such that the concentration of each component is no greater than 0.005 % weight/volume (0.05 micrograms/milliliter) in the chosen solvent. A typical chromatogram including components of the reference mixture on a typical non-polar fire debris column is shown in **Fig. 1**. A

typical chromatogram including components of the reference mixture on a fatty acid specific column is shown in **Fig. 2**.

6.9 Reference Oils and Fats—Oils and fats should be available for comparison and identification purposes.

6.9.1 Typically, FAMES derived from reference oils and fats are diluted approximately 1:200 in an appropriate solvent and derivatized using the same procedure that will be used on the debris and liquid samples. Depending on the column capacity and injection technique, derivatized oil and fat solutions can be concentrated to ensure detection of minor compounds.

6.10 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 reference mixture (see **6.8**). Optimize gas flow periodically.

7.2 Tune and Calibrate 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 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 Procedure

8.1 Only samples of appropriate dilution should be analyzed on a GC-MS system

8.2 Care must be taken to ensure that samples and extracts containing a small quantity of triglycerides and free fatty acids are not subjected to heat or evaporated to dryness prior to derivatization, as both of these may change the composition of the extract.

8.3 Analyze solvent blanks at least once each day that the instrument is used; maintain these analysis records. 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 sample is recommended but not required if studies demonstrate that the cleaning procedure is adequate to prevent carryover.

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