



ATF-LS-FD10 Extraction and Analysis of Vegetable Oils	Published Online: March 2018
Authority: Technical Leader	
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I. **Scope:** This policy and procedure guideline establishes a standard method for extracting, derivatizing, and analyzing vegetable oils encountered in neat liquids and fire debris samples. Vegetable oils contain triglycerides, which are fatty acids attached to a glycerol backbone. These are not volatile and will not be detected using typical fire debris headspace concentration techniques. However, if vegetable oils are suspected, a solvent extraction should be performed after routine ignitable liquid analysis. The extract is derivatized, which causes the triglyceride to break apart into fatty acids that are transesterified into fatty acid methyl esters (FAMES). The derivatized extract is analyzed on a specific GC column to identify the presence of FAMES. Co-extraction of interfering compounds may be a concern with some porous matrices using this technique. It should be noted that this procedure will also work for animal fats, such as butter, as these are similar in chemical composition to vegetable oils.

II. References:

ASTM E 2881 - Standard Test Method for Extraction and Derivatization of Vegetable Oils and Fats from Fire Debris and Liquid Samples with Analysis by Gas Chromatography-Mass Spectrometry

L Schwenk and M Reardon, "Practical aspects of analyzing vegetable oils in fire debris," *Journal of Forensic Sciences*, 54(4): 874-80, 2009.

A Gambrel and M Reardon, "Extraction, derivatization, and analysis of vegetable oils from fire debris," *Journal of Forensic Sciences*, 53(6):1372-80, 2008.

E Stauffer, "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*, 51:1016-32, 2006.

E Stauffer, "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*, 50:1091-1100, 2005.

E Stauffer, D Byron, and D Carpenter, Vegetable Oil Workshop, <http://www.swissforensic.org/publications/aafsvor/aafsvor.html>, American Academy of Forensic Sciences Annual Meeting, 2007.

ASTM E 1386 - Standard Practice for Separation and Concentration of Ignitable Liquid Residues from Fire Debris Samples by Solvent Extraction

ASTM E 1618 - Guide for Ignitable Liquid Residues in Extracts from Fire Debris Samples by Gas Chromatography-Mass Spectrometry

Material Safety Data Sheet for Pentane

Material Safety Data Sheet for Potassium Hydroxide (KOH)

Material Safety Data Sheet for Methanol

III. Apparatus/Reagents:

- A. Solvent - Pentane
- B. Derivatization reagent – approximately 2N KOH in methanol
- C. Drying agent - anhydrous sodium sulfate or equivalent
- D. Filter apparatus - free of extractable hydrocarbons
- E. Beakers or similar containers
- F. Commercially available test mixture, such as NuChek Prep #17AA, or equivalent containing the following FAMES: C8:0, C10:0, C12:0, C14:0, C16:0, C16:1, C17:0, C18:0, C18:1, C18:2, C18:3, C20:0, C22:0, C22:1, and C24:0.
- G. GC column - Supelco® SP-2380 or equivalent, 30 m x 0.20 mm, for analysis of FAMES
- H. Instrument - GC-MS
- I. Reference collection of various vegetable oils

IV. Safety Precautions:

Personal protective equipment including but not limited to safety glasses, gloves, and lab coat should be worn.

Pentane should be used in a fume hood and kept away from open flames and sparks.

V. Procedures

If ignitable liquid analysis is required, it shall be performed prior to analysis for oils and fats as the methods discussed below are destructive techniques. A fire debris sample (or sub-sample) or an aliquot of a liquid will be initially analyzed for ignitable liquid residues following ATF Fire Debris protocols. The same sample of fire debris (or a different sub-sample) or an additional aliquot of liquid is then extracted with an organic solvent, derivatized and analyzed on a GC-MS.

The following procedures are suggested for the extraction of neat liquids and debris samples and may be modified depending on sample material and size.

Neat liquids:

1. Place approximately one drop of liquid into a suitable container.
2. Add approximately 10 mL pentane.
3. Add approximately 0.5 mL of derivatization agent.
4. Seal container and shake.
5. Allow layers to separate.
6. Remove the top layer and filter as deemed necessary.
7. Analyze filtered extract on GC-MS.

Debris samples:

1. Open and examine the fire debris sample to determine that it is consistent with its description and perform routine ignitable liquid analysis, if required.
2. Extract sample (or suitable sub-sample) in its original container or in a disposable or new container by adding sufficient pentane to moisten the sample and then thoroughly mixing the pentane and debris. Simple rinsing of nonporous surfaces may not adequately separate residues.
3. Remove the extract from the debris. NOTE: If water is present, after removal of the extract from the debris, allow the pentane and water to separate. Decant the solvent layer into a clean

beaker and add a drying agent (such as anhydrous sodium sulfate) to the sample extract until the drying agent flows freely without clumping. The amount of drying agent added will vary depending on the amount of water present.

4. Transfer at least a 2 mL portion of the extract to a suitable container and add approximately 0.5 mL of derivatization agent. A portion of the pentane extract, prior to derivatization, may be preserved for further ignitable liquid analysis, if deemed necessary.
5. Seal container and shake.
6. Allow layers to separate.
7. Remove the top layer and filter as deemed necessary.
8. Analyze filtered extract on GC-MS.

Concentration – If the initial GC-MS results indicate that the sample needs to be concentrated, perform the concentration in containers free of extractable hydrocarbons (disposable or new containers). Place the container in a chemical fume hood and evaporate at room temperature. Compressed dry nitrogen can be used to accelerate evaporation. Extreme caution should be used if applying heat. Heating mantles are not recommended; however, a steam bath may be used to accelerate evaporation. Use of heat for concentration will cause the loss of the more volatile compounds present in the sample.

The identification of FAMES in liquids and fire debris is done using the retention time and spectral data compared with a known reference material, such as the quality control. **It should be noted that the presence of fatty acids in fire debris does not confirm the presence of vegetable oils.**

VI. Quality Control:

1. The instrument will be autotuned using perfluorotributylamine (PFTBA) no more than one week prior to casework analysis. The tune report shall be examined to insure that all parameters are within their normal expected range specified by manufacturer. Tune reports are maintained near the instrument
2. Each day the instrument is used for analysis, a quality control sample must be run. This quality control sample should be a commercially available test mixture, such as NuChek Prep #17AA, or equivalent containing the following FAMES: C8:0, C10:0, C12:0, C14:0, C16:0, C16:1, C17:0, C18:0, C18:1, C18:2, C18:3, C20:0, C22:0, C22:1, and C24:0. The suggested preparation is 100 mg of the test mixture in approximately 200 mL of methanol. However, this can be adjusted based on the sensitivity of the instrument. In cases where sequences carry over into the next day, the quality control sample will be considered still valid for the sequence. Additional quality control samples must be run for sequences continuing beyond the next day and each day thereafter. The data for the quality control sample will be maintained near the instrument and its use (including, at a minimum, the QC name and date prepared) will be documented in the case record.
3. For each batch of extracted samples, a system blank (or materials control) will be run prior to any samples. A system blank will be prepared the same way as the sample(s). This system blank insures that the instrument, solvent and method of analysis are free of contamination. A copy of system blanks will be maintained in the appropriate case jacket.
4. A solvent blank will be run between each case sample. This blank insures that no carryover from the previous sample exists.

5. A reference liquid containing fatty acids will be run contemporaneously with the case samples in order to verify the performance of the derivatization agent. If FAMEs are not observed in the reference liquid, a new derivatization agent will be prepared and all case samples will be reanalyzed once the agent's performance is verified.
6. When changes outside the acceptable parameters (as defined by manufacturer) occur in the "autotune" or the quality control sample (i.e. air leaks, decreased resolution, decreased sensitivity) check the following items as necessary:
 - a. Septum
 - b. Column nut
 - c. GC-MS interface nut
 - d. Injection port liner
 - e. Capillary column
 - f. MS source
7. FAMEs may be identified by retention time and spectral data on a GC-MS using a standard fire debris column and method (refer to the ATF Fire Debris GC-MS protocol). However, peak resolution is not sufficient for comparative purposes. If further information (such as possible type(s) of vegetable oil/fat or tendency to self-heat) or comparison is required, the extracts will be analyzed using a FAME-specific column.

Extract Storage - All extracts will be preserved with charcoal and returned with the evidence to the submitting agency.