Analysis of Animal Fats and Vegetable Oils

1. Scope

This procedure covers the extraction, derivatization, and analysis of vegetable oils encountered as neat liquids and in fire debris samples. Triglycerides and free fatty acids in vegetable oils are not suitable for extraction by passive adsorption-elution or separation and analysis by GC/MS due to their low volatility. Solvent extraction and derivatization of fatty acids to fatty acid methyl esters (FAMEs) are necessary for analysis. It should be noted that this technique will also work for animal fats as these are similar in composition to vegetable oils.

FAMEs are also present in biodiesel. Derivatization if not necessary to identify FAMEs in biodiesel. Biodiesel analysis is outlined in a separate procedure.

2. Terms and Definitions

FAME – fatty acid methyl ester

Triglyceride – an ester formed from glycerol and three fatty acid groups. Triglycerides are the main constituents of natural fats and oils.

Fatty acid – a carboxylic acid consisting of a hydrocarbon chain and a terminal carboxyl group, especially any of those occurring as esters in fats and oils.

3. References

ASTM E2881 *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*

4. Examination Procedures

4.1. Evidence Types

Fire debris or items suspected of containing oils and fats and liquid samples suspected of containing oils and fats.

4.2. Reagents and Chemicals

Hexane
Benzene

Carbon disulfide
Transesterification reagent, such as 3-(trifluoromethyl)phenyltrimethylammonium hydroxide (5% in MeOH)
Reference mixture containing at least the following FAMEs: C16:0, C18:0, C18:1, C18:2, and C18:3. A known transesterified vegetable oil standard may be substituted for this if it contains the necessary compounds.
Reference collection of various vegetable oils and animal fats

4.3. Reagent Preparation

Reference Mixture – Dilute reference mixture in hexane such that the concentration of each component is approximately 0.05 µg/mL. This concentration can be adjusted depending on the sensitivity of the instrument. Verify the solution is prepared properly by analyzing the solution by GC/MS. Retain chromatograms and mass spectra in Verification Log.

4.4. Instruments and Equipment

Gas Chromatograph/Mass Spectrometer (GC/MS)
Autosampler vials and caps
General laboratory glassware
Photographic equipment

4.5. Procedural and Chemical Precautions

Refer to the TBI Safety Manual for general safety requirements and hazard information regarding the use of reagents and solvents, and overall safety guidelines.

The transesterification process for the analysis of vegetable oils shall be performed in a fume hood.

Protective attire, including laboratory coat, mask, gloves, and eye protection should be used when working with clothing or bloodstained items.

Decontamination of a scientist's work area should be performed after each use, but shall be done after analyzing bloodstained items.

Hazardous chemicals shall be used in a chemical fume hood.

When handling concentrated acids, eye protection and laboratory coats shall be worn.

When diluting acids, always add acid to water.

When necessary, consult section and laboratory Safety Data Sheets (SDS) regarding any chemical used in the Microanalysis section.

Label all generated solutions and reagents with appropriate warning stickers.

4.6. Limitations

The identification of fatty acids does not necessarily mean that the fire was caused by self-heating.

This procedure is limited to conducive samples with visible stains or oily residues.

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

This is a destructive technique and whenever possible the entire sample should not be used for the procedure.

The identification of a specific type of oil (for example, olive, corn, or linseed) requires a quantitative analysis of the fatty acid esters and is beyond the scope of the procedure.

4.7. Procedure

Document submitted samples according to *Microanalysis Quality Assurance Policy*.

If appropriate and requested, perform analysis for ignitable liquid residues according to *Microanalysis Standard Operating Procedure Manual – Ignitable Liquid Residue Analysis* prior to analysis for oils and fats as the methods discussed below are destructive techniques.

4.7.1. **For Liquid Samples:**

Dissolve 25-50 µL (approximately 1 drop) of sample in 1 mL of benzene in a reaction vial or autosampler vial.

Add 1 mL of transesterification reagent and allow the mixture to stand at ambient temperature for 20-30 minutes.

4.7.2. **For Debris Samples or Items:**

Perform a visual examination of the item of evidence to isolate any areas of visible staining for extraction. If none are present, a representative portion of the item can be used for extraction.

Extract a portion of the stain in a clean container with sufficient hexane or carbon disulfide to thoroughly moisten the sample and mix for several minutes.

Separate the solvent from the debris.

Allow the solvent to evaporate to dryness (no heat). Reconstitute with approximately 1 mL of benzene.

Transfer benzene extract to reaction vial or autosampler vial.

Add 1ml of transesterification reagent and allow the mixture to stand at ambient temperature for 20-30 minutes. Filter or centrifuge if necessary.

4.7.3. **Analysis**

Prepare a negative control from solvent and reagent only.

Prepare a positive control using known reference oil in the same manner as liquid samples.

The FAME reference mixture shall be analyzed at the beginning and end of each auto sample run to ensure the system is performing properly. These chromatograms shall be enclosed in the case file.

Solvent blanks shall be analyzed before each sample to ensure there is no carryover on the column of the Gas Chromatograph/Mass Spectrometer. If contamination from the prior sample occurs, the affected sample(s) should be diluted (if necessary) and reanalyzed.

A nonpolar column may be used for general identification of FAMEs. However, the resulting peak resolution is not sufficient for evaluation of

self-heating tendency. If this information is requested, the extracts must be analyzed using a FAME-specific polar column.

Prepare instrument for analysis using the instrumental method FAME.M. Instrument parameters shall be retained as method files on the instrument computer and are electronically attached to each data file. The following are the critical parameters for this analysis. These may be adjusted as necessary to ensure proper peak resolution.

Oven Temperatures

Initial temp	175°C
Initial time	3.0 min
Ramp rate	10°C/min
Final temp	250°C
Hold time	2.0 min

Gas Type

Helium

Column Type

Polar: Cyanopropyl-based fatty acid stationary phase
Nonpolar: 100% polydimethylsiloxane or (5%-phenyl) methylpolysiloxane stationary phase

Length	20-30m
Internal diameter	0.2-0.25mm
Film thickness	0.1-0.5µm

Mass Spectrometer Parameters

Autotune file	atune.u
Mode	Scan
Solvent Delay	0.00min
Low Mass	45 amu
High Mass	350amu
MS Quad temp	150°C
MS Source temp	230°C

Injection

Volume	1.0 µL

Create auto sample sequence using instrument software. The sequence shall contain all GC checks, blanks, and samples in the order they are to be analyzed.

Place vials into auto sampler tray and check to ensure they are in the same order as listed in the sequence. Document this check in case notes. Also document in case notes that blank vials, wash bottles, and reference

mixture vial have been checked. Save sequence and begin the acquisition.

Once the acquisition is completed, re-check the sequence order of the sample vials and document in case file. Each sample shall be placed back in the container to which each is related.

4.8. Results and Interpretations

The identification of FAMES in liquids and fire debris is accomplished by comparing retention time and mass spectral data to known oil and fat reference materials.

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

1. Fatty acids were detected in the sample but no PUFAs were detected. These oils and fats have no tendency to self-heat.

2. Fatty acids were detected in the sample and low amounts of PUFAs were present. These oils and fats have a moderate tendency to self-heat.

3. Fatty acids were detected in the sample and high amounts of PUFAs were present. These oils and fats have a high tendency to self-heat.

It should be noted that combustion of vegetable oils is known to change the fatty acid content, and therefore a low amount of PUFAs does not rule out the possibility that a substance with a higher tendency toward self-heating was present prior to combustion.

The identification of an individual fatty acid in fire debris samples does not confirm the presence of vegetable oils or animal fats.

5. Reports

Analysis of the sample revealed the presence of fatty acids commonly found in, but not limited to, vegetable oils and animal fats.

Analysis of the sample did not reveal the presence of fatty acids commonly found in vegetable oils and animal fats.
If self-heating is an issue, the following may be added:

> The fatty acid composition indicates that this oil (or fat) has a very slight or low tendency toward spontaneous ignition. This does not eliminate the possibility that a substance with a higher tendency toward self-heating was present prior to the fire.
>
> The fatty acid composition indicates that this oil (or fat) has a moderate tendency toward spontaneous ignition. This does not eliminate the possibility that a substance with a higher tendency toward self-heating was present prior to the fire.
>
> The fatty acid composition indicates that this oil (or fat) has a high tendency toward spontaneous ignition. This does not necessarily imply that spontaneous ignition occurred.

The exact wording of the results on an official report will vary depending upon the case. At a minimum the presence or absence of oils and fats will be reported. Other qualifying information concerning the nature of the analysis or the evidence may be provided. Concluding statements may be added to clarify the results or show connections or non-connections between different items of evidence.