



TENNESSEE BUREAU OF INVESTIGATION

Forensic Services Division

Microanalysis Standard Operating Procedures Manual

Biodiesel Analysis

Analysis of Biodiesel Products

1. Scope

This procedure covers the analysis and identification of the fatty acid methyl ester (FAME) components of biodiesel products. Derivatization is not necessary to identify FAMES in biodiesel.

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 the procedure.

2. Terms and Definitions

FAME – fatty acid methyl ester

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.

Biodiesel – a fuel product made from fatty acid methyl esters (FAMES) or a blend of FAMES and petroleum products.

3. References

ASTM E2997 *Standard Test Method for Analysis of Biodiesel Products by Gas Chromatography-Mass Spectrometry*

4. Examination Procedures

4.1. Evidence Types

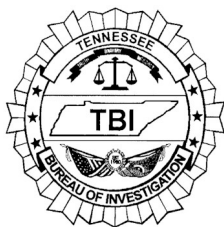
Fire debris or items suspected of containing biodiesel products and liquid samples suspected of containing biodiesel products.

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.

4.2. Reagents and Chemicals

Hexane

Carbon Disulfide



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Transesterification reagent, such as 3-(trifluoromethyl)phenyltrimethylammonium hydroxide (5% in MeOH)

ASTM 1618 Column Resolution Test Mixture

Reference Mixture containing 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.

4.3. Reagent Preparation

ASTM 1618 Column Resolution Test Mixture – Add 1 vial of the ASTM 1618 Column Resolution Test Mixture to a 10 mL volumetric flask and bring up to volume with carbon disulfide. Verify the solution is prepared properly by analyzing the solution by GC/MS. Retain chromatograms and mass spectra in Verification Log.

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.

Transesterified Reference Oil – Refer to the Microanalysis Analysis of Animal Fats and Vegetable Oils Standard Operating Procedure

4.4. Instruments and Equipment

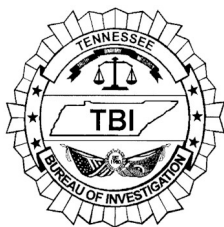
Gas Chromatograph-Mass Spectrometer (GC/MS)

Autosampler vials and caps

General laboratory glassware and plastic ware

Photographic equipment

4.5. Procedural and Chemical Precautions



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

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

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.

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

4.6. Limitations

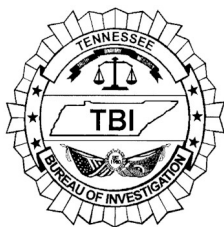
This procedure can be hampered by coincident extraction of interfering compounds present in the fire debris samples.

Depending on the extraction technique, this could be a destructive technique and whenever possible the entire sample should not be used for the procedure.

4.7. Procedure

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

Follow the Microanalysis Ignitable Liquid Residue Analysis Standard Operating Procedure in order to classify any petroleum product present.



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FAMES can be identified in debris samples using headspace extraction or solvent extraction techniques, so additional extraction of the debris is unnecessary. However, the extracts must be reanalyzed in the following manner:

The ASTM column resolution test mixture and FAME reference mixture (or transesterified reference oil) shall be analyzed at the beginning and end of each auto sample run to ensure the system is performing properly. These chromatograms are 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 sample should be diluted and reanalyzed.

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

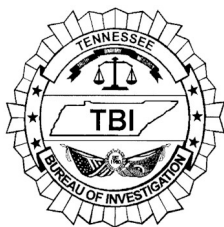
Polar: Cyanopropyl-based fatty acid stationary phase

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



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MS Source temp	230°C
<u>Injection</u>	
Volume	1.0 µL

Create auto sample sequence using instrument software. The sequence should 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 analysis run.

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

The identification of FAMES in liquids and fire debris is accomplished by comparing retention time data and mass spectral data to known reference materials. This can be either the FAME reference mixture or a transesterified reference standard. The presence of FAMES, with or without petroleum diesel, is an indication of biodiesel, as FAMES are not naturally occurring.

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 product.

5. Reports

Analysis of the sample revealed the presence of fatty acid methyl esters, which are indicative of a biodiesel product. Biodiesel products are ignitable liquids.

Analysis of the sample revealed the presence of a heavy petroleum distillate and fatty acid methyl esters. This combination is indicative of a biodiesel product. Biodiesel products are ignitable liquids.

Analysis of the sample did not reveal the presence of the fatty acid methyl esters indicative of biodiesel products.

The exact wording of the results may vary depending on the particular nature of an individual case.