12.0 Analysis of Animal Fats and Vegetable Oils

12.1 Scope - Fats are the main constituents of the storage cells in animals and plants, and are one of the important food reserves of the organism. We can extract these animal and vegetable fats (oils) to obtain cooking oils, greases, butter, etc. Chemically, fats are carboxylic esters derived from glycerol. Each fat is made up of glycerides derived from many different carboxylic acids. The proportions of the various acids vary from fat to fat and has its characteristic composition.

"With only a few exceptions, the fatty acids are all straight-chain compounds ranging from three to eighteen carbons and only acids containing even-numbered carbons are present in substantial amounts. The fatty acids may be saturated or unsaturated with one or more double bonds per molecule." (Morrison and Boyd, Organic Chemistry 4th ed., Allen and Bacon, Inc., Mass., 1983, pg. 1039.)

To determine the type of fat present, the analyst will compare fatty acid compositions between the questioned sample and known sample or laboratory standards. The most common method of analyzing fatty acid composition is by transesterification, a process where methanol replaces the alcohol group of the esters on the triglycerides. This produces three fatty acid methyl esters (FAMEs) and glycerol. The FAMEs can then be analyzed by GC/MS and samples compared to each other and to published data.

12.2 Terms and Definitions

Derivative - is a compound that is derived from a similar compound by some chemical or physical process.

Derivatization - the conversion of a chemical compound into a derivative (for an identification).

Transesterification - is the process of exchanging the organic group R" of an ester with the organic group R′ of an alcohol.

12.3 References –

Derivatization of Corn Oil for Analysis by Gas Chromatography”, Application Note 123, Supelco, Sigma-Aldrich, 1997


12.4 Examination Procedures -

12.4.1 Evidence Types – Animal fats and vegetable oils from a crime scene or from clothing or any material which may have come in contact with animal fats and vegetable oils.

12.4.2 Reagents and Chemicals -
1. Sulfuric Acid/Methanol Reagent
2. Methanolic HCl
3. Instant Methanolic HCl kit (Alltech Associates)
4. Boron Trifluoride/Methanol (14% w/v)
5. METH-PREP II Transesterification Reagent

12.4.2.1 Reagent Preparation

Sulfuric Acid/Methanol Reagent - Mix together 90mL methanol, 30mL benzene, and 1mL concentrated sulfuric acid.

Methanolic HCL - Slowly add 3 ml HCl to 97 ml methanol to produce a 3% solution.

Instant Methanolic HCl Kit - Dropwise add 2.8 ml acetyl chloride to 56 ml methanol to produce a 3% solution.

12.4.3 Procedural and Chemical Precautions – See Section 6.0 of this Manual for all safety precautions.

12.4.4 Limitations –
Procedure is limited by amount of material on an item of evidence. Other extraneous materials may extract from an item of evidence and overwhelm or mask the oil or fat extracted. The determination of the type of vegetable oil or animal fat is limited.

12.4.5 Procedure -

If the oil or fat must be extracted from an item of evidence, only a portion of the item will be extracted. A description and/or photograph of the extracted item will be included in the case notes.

Place the item over or in a clean beaker or tray and wash with an appropriate solvent under a hood.

Collect the liquid and evaporate to dryness or an appropriately small volume under a hood. If the sample is allowed to go to dryness, reconstitute the residue in the solvent appropriate for the derivatization.

A known positive control and a negative control will be prepared and analyzed along with the unknown sample.

Derivatization – There are a variety of transesterification procedures that are applicable to forensic samples. The results of the transesterification will not vary between the different procedures.


Mix fat or oil and excess of reagent in capped reaction vial. Heat approximately 30 minutes and agitate. (Optional) Allow to stand overnight.

Wash the mixture 1-5 times with water to remove the methanol and acid.
Extract with a small amount of diethyl ether.

Prepare ether extract for Gas Chromatograph/Mass Spectrometer analysis.

**Methanolic HCL** (“Derivatization of Corn Oil for Analysis by Gas Chromatography”, Application Note 123, Supelco, Sigma-Aldrich, 1997.)

Mix a small volume of fat or oil, 1ml of 3N methanolic HCl, and 1ml hexane in a capped reaction vial.

Heat approximately 10-15 minutes and cool to ambient temperature.

Add 1ml of water and 1ml of hexane. Agitate and allow phases to separate.

Prepare upper layer for Gas Chromatograph/Mass Spectrometer analysis.

Note: The addition of 250 ul of 2,2-dimethoxypropane in step 1 will drive the reaction to completion. In normal casework this is not necessary since it is not a quantitative analysis.


Add slowly one ampoule (2.8ml) of acetyl chloride to 56ml of methanol to produce a 3% solution.

Add 5ml of this solution to approximately 50mg of sample and allow the mixture to react at ambient temperature for 15 minutes.

Evaporate to dryness and reconstitute in solvent of choice for Gas Chromatograph/Mass Spectrometer analysis.

Place up to 200mg of sample in a large test tube or capped reaction vial.

Add 5ml of boron trifluoride/methanol reagent and heat to boiling for 2 minutes.

Cool to ambient temperature and add 30 ml of petroleum ether and 20ml of water.

Extract with vigorous shaking.

Remove petroleum ether layer and reduce volume for Gas Chromatograph/Mass Spectrometer analysis.


Dissolve up to 50mg of sample in 1ml of benzene.

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

Prepare solution for Gas Chromatograph/Mass Spectrometer analysis.

**Analysis.** The samples may be analyzed on a gas chromatograph/mass spectrometer using a variety of different columns. The following is a guide to the differences between classes of columns.

- **Mid-Polar, Polyethylene Glycol or WAX Columns** – These columns should elute the FAMEs primarily by carbon chain length and secondarily by the number of double bonds. There should be minimal overlap in elution order among FAMEs having different chain lengths. These columns suffer from the fact that some nutritionally important FAMEs do coelute.

- **Polar, Cyansilicone Columns** – Cyansilicone columns are highly polar phases that allow the separation of polar compounds with close boiling points. These columns can resolve cis, trans isomers and olefinic positional isomers of
the FAMEs. However, there will be crossover in elution sequence among FAMEs of different chain length and degree of saturation.

- Nonpolar Columns – These columns elute FAMEs in order of boiling point. Unfortunately there is considerable overlap in elution order among unsaturated acids of the same chain length. So some of the nutritionally important FAMEs cannot be fully resolved. (“Analyzing Fatty Acids By Capillary Gas Chromatography”, Bulletin 855B, Supelco, Sigma-Aldrich, 1998.)

Prepare instrument for analysis using instrumental method “FAME.m”. Instrument parameters are retained as method files on the instrument computer and are electronically attached to each data file. Following are the critical parameters for this analysis.

**Oven Temperatures**
- Initial temp 200°C
- Initial time 3.0 min
- Ramp rate 10°C/min
- Final temp 250°C
- Hold time 2.0 min

**Gas Type**
- Helium – constant flow mode

**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

The inlet may be set for splitless injection (0.05 min purge time, 100mL/min purge flow) or split injection (200:1 split ratio) depending on the concentration of the sample.

A solvent blank must be analyzed prior to analyzing each sample. The ASTM test mixture must also be analyzed with each case.
Known vegetable oil or animal fat standards shall be prepared from a submitted standard or from a standard in the TBI reference library for comparison purposes. All data generated during the analysis must be retained in the case file.

Compare the results of the instrumental testing of the known and unknown samples and report the findings. Identification is based on peak-to-peak comparison and various peak identifications.

12.5 Instruments and Equipment -
1. Graduated cylinders
2. Beakers and/or Erlenmeyer Flasks
3. Hot plate
4. Stoppers
5. Test tubes and/or GC vials
6. Glass and/or plastic centrifuge tubes or reaction vials
7. Gas Chromatograph
8. Gas Chromatograph-Mass Spectrometer
9. Photographic equipment with accessories

12.6 Measurement Traceability – These examinations and comparisons are qualitative techniques and as such do not utilize measurements that will have a significant effect on the outcome of the analysis.

12.7 Reference Materials –
1. Known animal fats and plant oils.

12.8 Reports –
The following are possible results concluded from the examination:

Analysis of the sample revealed the presence of vegetable oil.

Analysis of the sample revealed the presence of an animal fat.

Analysis of the sample did not reveal the presence of vegetable oil.

Analysis of the sample did not reveal the presence of an animal fat.

The exact wording of the results on an official report will vary depending upon the case. At a minimum the presence or absence
of an animal fat or vegetable oil 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.