



AOCS Ce 1k-09  
Direct Methylation of Lipids for the  
Determination of Total Fat, Saturated, *cis*-  
Monounsaturated, *cis*-Polyunsaturated and *trans*  
Fatty Acids by Gas Chromatography

Presentation to  
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## Advantages of AOCS Ce 1k-09

- No sample transfer for precise measurements
- Low levels of solvents
- 15 minute saponification
- MEs are in one vessel

# Definition for Procedure A: Alkaline Only

- Fat or oil is simultaneously alkali hydrolyzed (sodium hydroxide in methanol) and extracted with an organic solvent (*n*-hexanes or *n*-heptane) from its matrix and methylated with boron trifluoride in methanol as the catalyst. The fatty acid methyl esters (FAMES) are quantitatively determined by capillary gas chromatography (AOCS method Ce 1h-05) with 21:0, triacylglycerol (TAG), as the internal standards.
- Total fat is calculated as sum of individual fatty acids expressed as triglyceride equivalents.
- Saturated, *cis*-monounsaturated, *cis*-polyunsaturated, and *trans* fatty acids are calculated as sum of respective fatty acids.



# SCOPE for Procedure A

- This method should be applicable for a majority of matrices with vegetable oil lipids present (e.g., foodstuffs, beverages, tissues, and oils).
- It is not applicable to dairy or ruminant fats and marine or long chain polyunsaturated oils, or products containing micro-encapsulated oils.
- The method has been used on a variety of matrices with success. On new matrices (since all matrices could not be evaluated), it may be worthwhile to examine the method against appropriate AOCS or AOAC (e.g., AOAC 996.06) reference methods.
- Use the appropriate grinding technique for homogenization of the sample when necessary. Many samples with high moisture contents do not require drying before analysis. However, samples that require drying should have the precaution taken to prevent the fat/oil in the samples from becoming oxidized if any other method (than freeze drying) is used for drying.
- The moisture content can be used in a back calculation to determine the total fat, saturated, *cis*-monounsaturated, *cis*-polyunsaturated, and *trans* fatty acid contents.
- See AOCS method Ce 1h-05 for GC conditions and calculations of total fat, saturated, *cis*-monounsaturated, *cis*-polyunsaturated, and *trans* fatty acid contents.



# DEFINITION for Procedure B

- Fat or oil is released from a matrix utilizing an acid digestion (hydrochloric acid in methanol). The lipid material is then simultaneously alkali hydrolyzed (sodium hydroxide in methanol) and methylated (with boron trifluoride in methanol as the catalyst).
- The fatty acid methyl esters (FAMES) are quantitatively determined by capillary gas chromatography (AOCS methods Ce 1j-07, Ce 1h-05 or Ce 1i-07) with 13:0, 21:0, or 23:0 triacylglycerols (TAGs), respectively, as the internal standards.
- The appropriate GC method is chosen basis major fat source in food matrix or health claim to be made. Typically, AOCS Ce 1h-05 is used for vegetable oils, AOCS Ce 1i-07 is used for marine and PUFA oils, and AOCS Ce 1j-07 is used for dairy and ruminant fats.
- Total fat is calculated as sum of individual fatty acids expressed as triglyceride equivalents. Saturated, *cis*-monounsaturated, *cis*-polyunsaturated, and *trans* fatty acids are calculated as sum of respective fatty acids.
- Theoretical Correction Factors (TCFs) are used to quantitate all saturated and monounsaturated fatty acids and polyunsaturated fatty acids (PUFA) of 18 carbons. TCFs are also used for fatty acids, which lack standards. Empirical Correction Factors (ECFs) are used for long chain PUFA of 20 carbons or more and three or more double bonds of which standards are readily available.

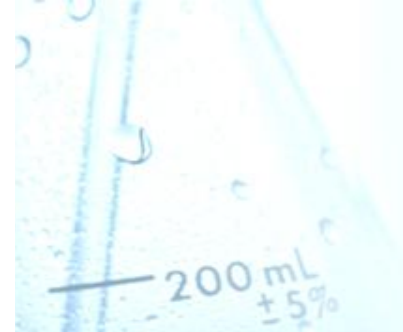


# Scope for Procedure B

- This method should be applicable for a majority of matrices with lipids present (e.g., food stuffs, beverages, tissues, and oils).
- Specifically, encapsulated oils will benefit as a number of these samples require an acid digestion to break down the encapsulant.
- The method has been used on a variety of matrices with success. On new matrices (since all matrices could not be evaluated), it may be worthwhile to examine the method against appropriate AOCS or AOAC (e.g., AOAC 996.06) reference methods.
- Use the appropriate grinding technique for homogenization of the sample when necessary.
- Many samples with high moisture contents do not require drying before analysis. However, samples that require drying should have the precaution taken to prevent the fat/oil in the samples from becoming oxidized if any other method (than freeze drying) is used for drying.
- The moisture content can be used in a back calculation to determine the total fat, saturated, *cis*-monounsaturated, *cis*-polyunsaturated and *trans* fatty acids contents. See AOCS methods Ce 1h-05, Ce 1i-07 or Ce 1j-07 for GC conditions and calculations of total fat, saturated, *cis*-monounsaturated, *cis*-polyunsaturated and *trans* fatty acid contents.

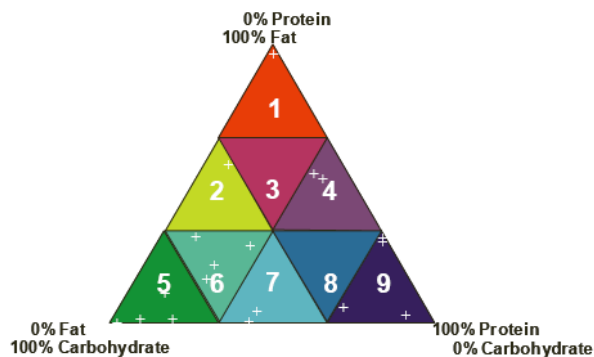
# Collaborative Study Samples

Orange Juice fortified with DHA/EPA	Chocolate cake mix
Liquid infant formula	Whole egg powder
Extruded petfood, high protein/high fat	Full-fat soy flour
Canned meat or catfood	Encapsulated DHA/EPA (Ocean Nutrition)
Butter	Creamy ranch dressing
Yogurt	Potato chips
Cheese powder	Oatmeal cookies
Evaporated or Condensed Milk	Frozen CHEESE pizza (grind whole then split)
Peanut Butter or Baking Chocolate	Cheese powder duplicate
Dry cereal fortified with flax	Peanut Butter or Baking Chocolate



<u>Food Triangle Sector</u>	<u>SRM Coverage of Food Triangle</u>
1	SRM 1563 Cholesterol and Fat-Soluble Vitamins in Coconut Oil
2	SRM 2384 Baking Chocolate
3	SRM 2387 Peanut Butter
4	SRM 1546 Meat Homogenate ( <i>recently completed</i> )
5	SRM 2383 Baby Food Composite ( <i>recently completed</i> )
6	SRM 1846 Infant Formula ( <i>recently completed</i> ) SRM 1548a Total Diet ( <i>recently completed</i> ) SRM 1544 Fatty Acids and Cholesterol in a Frozen Diet Composite
7	SRM 1566b Oyster Tissue ( <i>recently completed</i> ) SRM 1570a Trace Elements in Spinach Leaves SRM 2385 Spinach
Junction of 8, 9	SRM 1974a = Mussel Tissue
Junction of 4,8,9	SRM 1946 Lake Superior Fish Tissue SRM 1947 Lake Michigan Fish Tissue

Fig. 1. Populated AOAC food triangle with food matrices chosen by stakeholder







# Contact Information

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