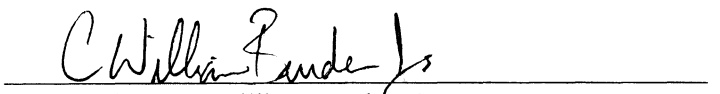
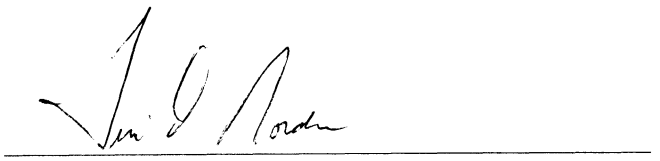
WI NO.: FAME_1 (Filename: FAME.r02)		Page 1 of 11
Title: Determination of Fatty Acid Methyl Esters in Fats and Oils by GC		
Lab: USDA,GIPSA,FGIS,TSD,ARTS	Program: Fatty Acid Reference Program	
Revision: 2	Replaces: 1	Effective: 10/07/02



10/2/02

Approved By: C. William Burden, Jr. Date
Chemist

USDA/GIPSA
10383 N. Ambassador Drive
Kansas City, MO 64153
(816) 891-0437



10/2/02

Approved By: Timothy D. Norden Date
ARTS Branch Chief

USDA/GIPSA
10383 N. Ambassador Drive
Kansas City, MO 64153
(816) 891-0470

Changes From Previous Revision

1. In section 5. Equipment:
   Added: Analytical balance (i), Digital pipetter (j and k), and Flow meter (l).

2. In section 6. Materials:
   Added: Compressed air (h), Soybean oil standard (i), Sunflower oil standard (j), GC Standard-37 Component FAME mix (k), Disposable transfer pipettes (m), and Sodium sulfate (n).

3. In section 7. Preparation of the Standard:
   Added a reference to the Sunflower oil standard.

4. In section 8. Procedure:
   Replaced the references to using a graduated pipette with an adjustable digital pipetter.

5. In section 9. Instrument Parameters:
   Changed the formatting of the tables and included more comprehensive information regarding the instrument's parameters.

6. In section 11. Calculation:
   Completely redone with detail added regarding the use of Microsoft Excel, Relative Retention Times, Area Percentages, tolerance criteria, and actions to take if the tolerances are violated.

Table of Contents

1. Purpose and Scope of Application

The purpose of this Working Instruction (WI) is to establish the operational parameters, methodology, and requirements for the quality assurance and acceptability of data in the determination of the fatty acid composition in fats and oils.

2. Analyst Qualifications and Responsibilities

The analyst(s) will receive proper training in the conduct of this WI and will follow the WI as written. The Supervisory Chemist (or Project Leader) is responsible for ensuring the WI is followed and modified as necessary or appropriate. All revisions to this WI must be approved by the Chief of the Analytical, Reference and Testing Services Branch, prior to implementation.

3. References

- American Oil Chemists Society (AOCS) Ce 1-62.
- AOCS Method Ce 2-66.
- International Union of Pure and Applied Chemistry (IUPAC) Standard Method for Analysis of Oils, Fats and Derivatives. Blackwell Scientific Publications 7th Edition (1987); IUPAC Method 2.303.

4. Safety and Hazardous Waste

Refer to the Technical Services Division Chemical Hygiene Plan.

5. Equipment

a. Round bottom boiling flask (50 ml) with T24/40 outer neck.

b. Water-cooled condensers, Liebig or West design, 20 to 30 cm jacket, with T24/40 inner joint.

c. Storage Vial (2 ml volume size).

d. Crimp cap auto sampler vial tool.

e. Steam bath.

f. Hewlett-Packard #5890 Series II Gas Chromatograph with Flame Ionization Detector.

g. Autosampler vials and crimp caps.

h. Supelco-SPB-PUFA column (30 m x 0.25 mm ID, 0.20 µm film) installed in Port A of the GC.

i. Analytical balance

j. Digital pipetter, adjustable volume, 0.5 ml - 5.0 ml.

k. Digital pipetter, fixed volume, 0.05 ml.

l. Flow meter.

6. Materials

a. Boron-trifluoride (BF_3) methanol reagent (125 g BF_3 per liter of methanol).

b. Methanolic Base, (NaOH, 0.5*N* in methanol).

c. Sodium chloride (NaCl) saturated solution in water (360 g/L).

d. Heptane, gas chromatographic grade.

e. Helium, chromatographic grade (minimum purity 99.9995%).

f. Nitrogen, research grade (minimum purity 99.9995%).

g. Hydrogen, research grade (minimum purity 99.9995%).

h. Compressed Air, UHP/Zero grade.

i. Soybean Oil Standard (Supelco 47122).

j. Sunflower Oil Standard (Supelco 47123).

k. GC Standard - 37 Component FAME mix (Supelco 47885-U).

l. Boiling Beads (~3 per sample).

m. Disposable transfer pipettes.

n. Sodium sulfate, anhydrous, ACS.

7. Preparation of the Standard

a. The sunflower oil standard (and/or soybean oil standard if sample is from soybeans) will be prepared using the same procedure as the sample. A solvent blank (heptane) and the sunflower oil standard (and/or soybean oil standard) will be used with each analysis run.

b. The GC standard (FAME Mix) will be run for comparison as is.

8. Procedure

a. Using the analytical balance and a disposable transfer pipette, weigh 0.100 to 0.200 grams of sample into a round bottom boiling flask. Add a few boiling beads to the flask.

b. Using the adjustable digital pipetter, add 4 ml of methanolic base to the flask, connect the flask to a condenser, and boil for seven minutes on the steam bath.

c. Using the adjustable digital pipetter, add 5 ml of BF_3 methanol reagent through the condenser and continue boiling for two minutes.

d. Using the adjustable digital pipetter, add 5 ml of heptane through the condenser and continue boiling for one minute. Remove the flask-condenser assembly from the steam bath and allow the flask to cool to room temperature.

e. Remove the flask from the condenser and add enough saturated salt solution to float the heptane layer to the neck.

f. Using a disposable transfer pipette, transfer approximately 2 ml of the top layer to a storage vial containing approximately 50 to 100 mg of anhydrous sodium sulfate and allow to dry for 10 minutes, or until the solution is clear.

g. Using the digital pipetters, make a 1:20 dilution of the dry heptane solution by transferring 0.05 ml of the sample and 0.95 ml of heptane to an autosampler vial. Crimp a cap onto the vial.

9. Instrument Parameters

Run Time Checklist

Pre-Run Cmd/Macro	Off
Data Acquisition:	On
Standard Data Analysis:	On
Customized Data Analysis:	Off
Save GLP Data:	Off
Post-Run Cmd/Macro:	Off
Save Method with Data:	On

Injection Source and Location

Injection source:	GC Injector
Injection Location:	Front

7673 Injector

Front Injector:

Sample Washes	3
Sample Pumps	3
Injection Volume	1.0 microliter
Syringe Size	5.0 microliters
On Column	Off
Nanoliter Adapter	Off
PostInj Solvent A Washes	3
PostInj Solvent B Washes	3
Viscosity Delay	0 seconds
Plunger Speed	Slow

Back Injector: No Parameters specified

Oven\Det

Runtime (min): 64.5

Zone temperatures:

	State	Setpoint
Inl. A	On	250 C.
Inl. B	Off	50 C.
Det. A	On	260 C.
Det. B	Off	50 C.
Aux.	Off	50 C.

Oven Zone:

Oven max	220 C.
Equib Time	0.50 Min.
Oven State	On
Cryo State	Off
Ambient	25 C.
Cryo Blast	Off

Oven Program:

	Setpoint
Initial Temp.:	50 C.
Initial Time:	2.00 Min.

Level	Rate (C/min.)	Final Temp. (C)	Final Time (min)
1	4.00	220	20.0

Purge Valve Settings

Purge A/B	Init Value	On Time (Min.)	Off Time (Min.)
A (Valve 3)	On	0.00	0.00
B (Valve 4)	On	0.00	0.00

A – Splitless Injection: No
B – Splitless Injection: No

Valves/Relays Information

Initial setpoints: 5890 Valves:

Valve 1:	Off
Valve 2:	Off
Valve 3 (Purge A):	On
Valve 4 (Purge B):	On

Detector Information

Detector A:

Type	FID
State	On

Signal Information

Save Data: Signal 1

	Signal 1	Signal 2
Signal	Det. A	Testplot
Data rate	20.000 Hz.	5.000 Hz.
Peakwidth	0.013 min.	0.053 min.
Start Time	0.00 min.	0.00 min.
Stop Time	650.0 min.	650.00 min.

Integration Events

Results will be produced with the enhanced integrator.

	"Event"		"Event_FID"	
Event	Value	Time	Value	Time
Initial Slope Sensitivity	1.000	Initial	1000.000	Initial
Initial Peak Width	0.040	Initial	0.040	Initial
Initial Area Reject	1.000	Initial	100.000	Initial
Initial Height Reject	1.700	Initial	500.000	Initial
Initial Shoulders	Off	Initial	Off	Initial

Calibration Table

Calculate	Area percent
Rel. Reference Window	5.000%
Abs. Reference Window	0.000 min
Rel. Non-ref. Window	5.000%
Abs. Non-ref. Window	0.000 min
Uncalibrated Peaks	Not reported
Partial Calibration	Yes, identified peaks are recalibrated
Correct All Ret. Times	No, only for identified peaks
Curve Type	Linear
Origin	Included
Weight	Equal

Recalibration Settings:

Average Response	Average all calibrations
Average Retention Time	Floating Average New 75%

Calibration Report options:

Printout of recalibrations within a sequence:
Calibration Table after Recalibration
Normal Report after Recalibration
If the sequence is done with bracketing:
Results of first cycle (ending previous bracket)

10. GC Instructions for Fatty Acid Methyl Ester Analysis

This procedure assumes that the Supelco SPB-PUFA column is installed in Port A of the GC.

a. If an "Instrument 2" session has not already been started on the GC Chemstation, double-click on the "Instrument 2 Online" icon.

b. If it is not already loaded, load the method, "Soybean.mth," by selecting "Load" from the Method menu.

c. Confirm that the "COLUMN HEAD PRESSURE" on Injection Port A is set at exactly 25 psi and that the flow through this port is reading 100 ml/min on the GC's display. Adjust the flow, if necessary, using the "TOTAL FLOW" knob.

d. Connect the flow meter to the FID outlet. The meter should read about 2.1-2.4 ml/min.

e. Open the "AUX GAS" valve and set the total flow to 32 ± 1 ml/min. You may need to turn the inner screw to set this flow.

f. Open the "HYDROGEN" valve and set the total flow to 62 ± 3 ml/min. by adjusting the hydrogen pressure at the regulator behind the GC.

g. Turn off the "HYDROGEN" valve and open the "AIR" valve. Adjust the total flow to 400 ± 5 ml/min. using the regulator behind the GC. Remove the flow meter from the FID outlet.

h. Open the "HYDROGEN" valve again and push the "FID IGNITOR" button on the GC detector panel. You will hear a faint "popping" noise. Check to see if the flame is lit by looking for water condensation above the detector using a small shiny object.

i. Adjust the on-screen attenuation to "0" and wait for the baseline to become flat (approximately 1 hour).

j. Set up the batch by entering the sequence information in "Sequence Parameters" and "Sequence Table" under the GC Chemstation "Sequence" menu. Save and print the sequence when you are finished.

k. Record the date, operator's name, batch name, and GC parameters in the logbook.

l. Choose "Run Sequence" from the GC Chemstation "Run" menu to start analyzing the samples.

11. Calculation

1. Use the data sheet supplied with the 37 Component FAME Mix to identify its peaks from the chromatographic assay.

2. Enter the Retention Time (RT) for each of the 37 constituents into the Excel spreadsheet. The spreadsheet will automatically calculate the Relative Retention Time (RRT) of each using oleic acid as the basis.

3. Review the chromatogram for the solvent blank to confirm the purity of the heptane. If the chromatogram shows evidence of contamination (peaks), repeat the injection using another portion of heptane.

4. Use the data sheet supplied with the sunflower and/or soybean standard to identify its constituents' peaks and corresponding area percentages.

5. Enter the RT and Area of these constituents into the spreadsheet. Their RRT's must all be within 0.005 of the RRT's for the same constituents in the FAME Mix. If they are not, investigate for a possible cause(s), correct if found, and repeat the analysis.

6. The Area Percent of each constituent (expressed as % methyl ester) in the sunflower and/or soybean oil standard is calculated by the spreadsheet using the following equation:

$$\%\ methyl\ ester = \frac{area\ of\ methyl\ ester}{total\ methyl\ ester\ area} \times 100$$

7. The Area Percent of each constituent in the sunflower and/or soybean oil standard must be within 10% of the values provided on the Supelco Certificate of Analysis. If they are not, investigate for a possible cause(s), correct if found, and repeat the analysis.

8. Enter the RT and area of the constituents for each sample into the spreadsheet. Again, their RRT's must all be within 0.005 of the RRT's for the same constituents in the FAME Mix. If they are not, investigate for a possible cause(s), correct if found, and repeat the analysis.

9. Report the results to the nearest tenth of a percent.