SPECTRA ANALYSIS

APPLICATION NOTE 005

IR Analysis of Fatty Acid Methyl Esters

Analysis of Fatty Acid Methyl Esters (FAMEs) is an important tool in the characterization of fats and oils and in the determination of total fat and trans-fat content in foods.

To prepare samples for analysis, fats are extracted, saponified and methylated to produce the methyl esters. FAMEs are both more volatile and more chemically stable than the corresponding oils, which allow analysis and quantitation by Gas Chromatography. The Infrared Spectra of FAMEs yield information about the chain length and the degree of unsaturation.

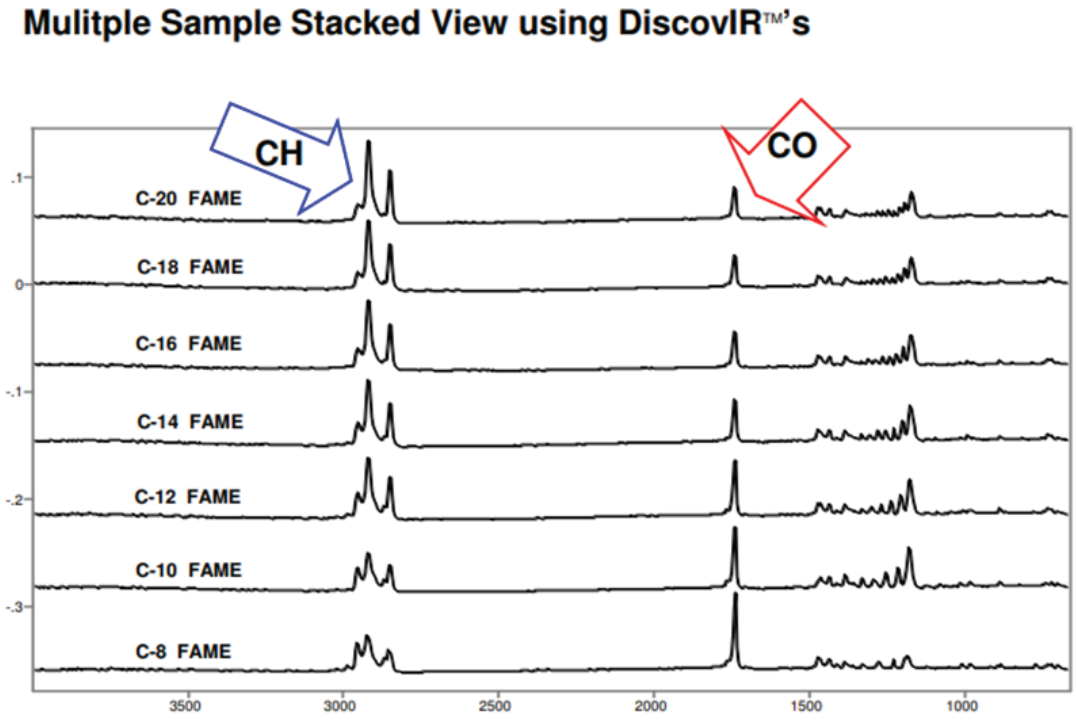

Fig. 1 The chain length of the FAME can be determined from the ratio of the heights of the Methylene absorbance peak (approx. 2920) and the Carbonyl peak (approx. 1740). As chain length increases, the CH peaks grow, and the ratio to the height of the CO peak increases.

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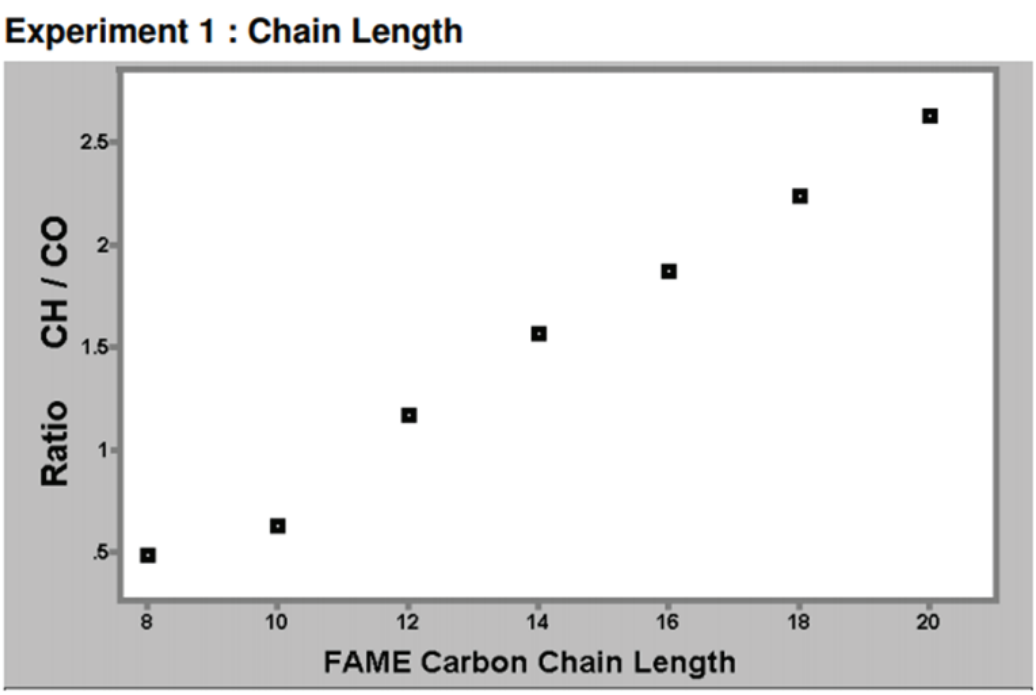

Fig. 2 When the CH/CO height ratio is plotted for standard FAMEs, it is easy to read off the number of Carbons in the Chain. Unknowns can be plotted on the same curve to determine chain length.

Sample Conditions for Experiment 1:

Sample:	Alltech L206 Fatty Acid Methyl Ester Mixture
Conc:	0.57 mg/mL of each component in hexane
Injection:	1 uL using split/splitless injector, split 40:1
Column:	DB5, 20-meter x 0.32-mm ID, 0.25-*um* film
Conditions:	Helium carrier, 1 mL/min
	Temp program: 70^0C for 3 min, 6^0/min up to 200^0

Injector, transfer line, restrictor tip all at 250^0C
Sample window -100^0C

Assessing the Degree of Unsaturation

As FAME chain lengths get longer, it can be difficult for other methods to determine the number and position of double bonds present in the molecule. Although there is a wealth of information to be discerned in IR spectra of these compounds, two main features are apparent when comparing saturated chains to those containing double bonds.

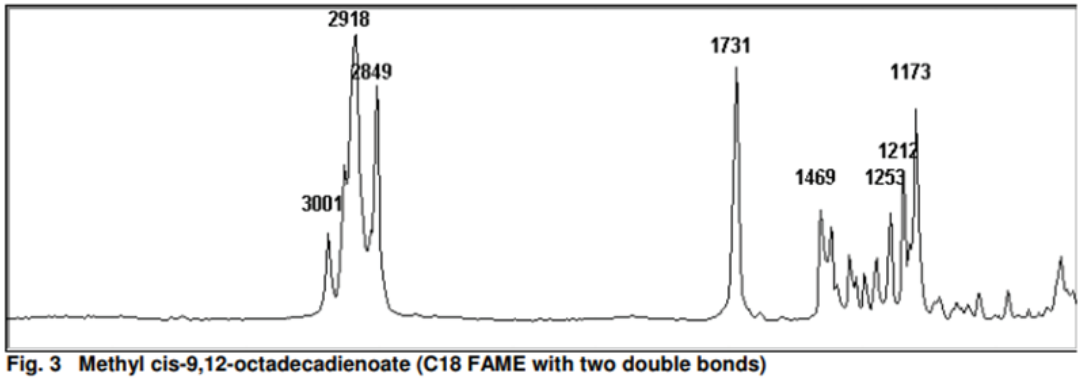

Fig. 3 Methyl cis-9,12-octadecadienoate (C18 FAME with two double bonds)

Sample Conditions for Experiment 2:

Sample : Alltech AOCS Oil Reference Mixture 03
Conc: 50 mg/mL in hexane
Injection: 1 uL using split/splitless injector
40:1 split ratio
Column: DB5, 20-meter x 0.32-mm ID, 0.25-*um* film
Conditions: Helium carrier, 1 mL/min
Temperature program: 70^0 for 3 min, 6^0/min up to 200^0
Injector, transfer line, and restrictor tip all at 250^0
Sample window -100^0

FAME Standard Compounds:

1. methyl tetradecanoate, 1% (C14)
2. methyl hexadecanoate, 4% (C16)
3. methyl cis-9,12,15-octadecatrienoate, 3% (C18)
4. methyl cis-9,12-octadecadienoate, 15% (C18)
5. methyl cis-9-octadecenoate, 45% (C18)
6. methyl octadecanoate, 3% (C18)
7. methyl eicosanoate, 3% (C20)
8. methyl cis-13-docosenoate, 20% (C22)
9. methyl docosanoate, 3% (C22)
10. methyl tetracosanoate, 3% (C24)

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Experiment 2 : Degree of Unsaturation

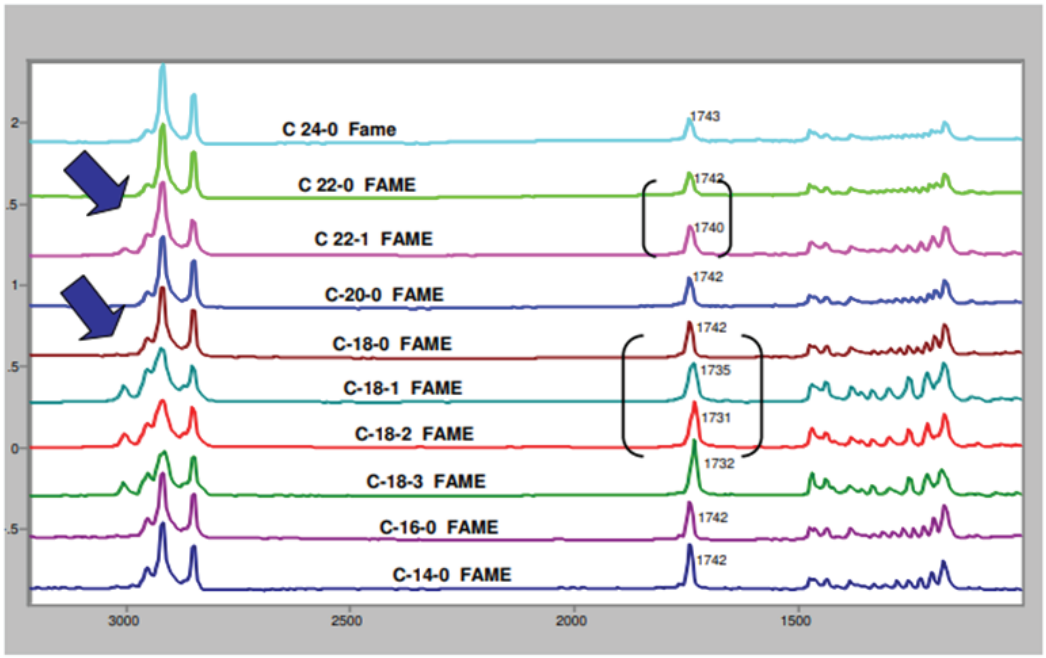

Fig. 4 FAME Series C-14 to C-24

When a series of FAME compounds is analyzed, the spectral features which distinguish the unsaturated species become clear. In the 18-Carbon chains shown above, with 1, 2 and 3 double bonds respectively, the appearance of the olefinic group above 3000 and the downward shift of the carbonyl are easy to see in comparison to the other chains with no double bonds.

The 22-Carbon chain also shows the olefinic peak, but interestingly, the carbonyl shift is less pronounced due to conformational constraints which reduce the interaction between the C=C bond and the C=O group. The collected spectra can be interpreted by inspection or compared by to a library of similar compounds. Libraries are available both in the public domain and through commercial vendors. The software included in the DiscovIR system makes library searches powerful yet simple to perform.