

APPLICATION NOTE 006

Infrared Analysis of Fatty Acid Methyl Esters by Direct Deposition after Gas Chromatography

Chromatographic 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 acids, which allow analysis and quantitation by Gas Chromatography.

Infrared analysis of FAMES is also an important tool used commonly to identify trans- and cisomers, among other features. DiscovIR combines the power of both methods in an automated approach that yields important structural information for each compound in addition to retention time comparison to standards.

Separation of FAME Standards by GC using DiscovIR

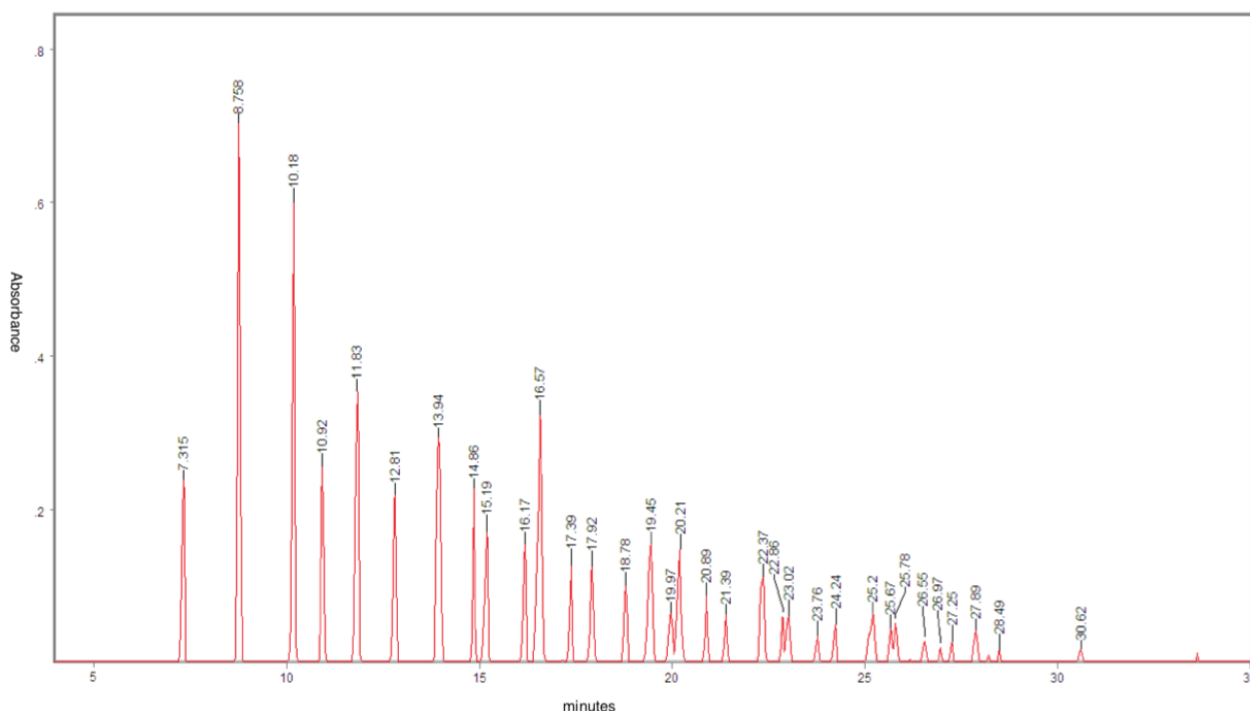


Fig. 1 Peak Chromatogram of a 37-Component FAME mixture.

In a peak chromatogram, the maximum absorbance detected anywhere in the mid-IR spectrum is plotted for every point in time. Note the peak shapes that show very little band spreading. Refer to **Table 1** for Chromatographic conditions and **Table 2** for standard mixture components.

Structural Characterization of FAMES by IR

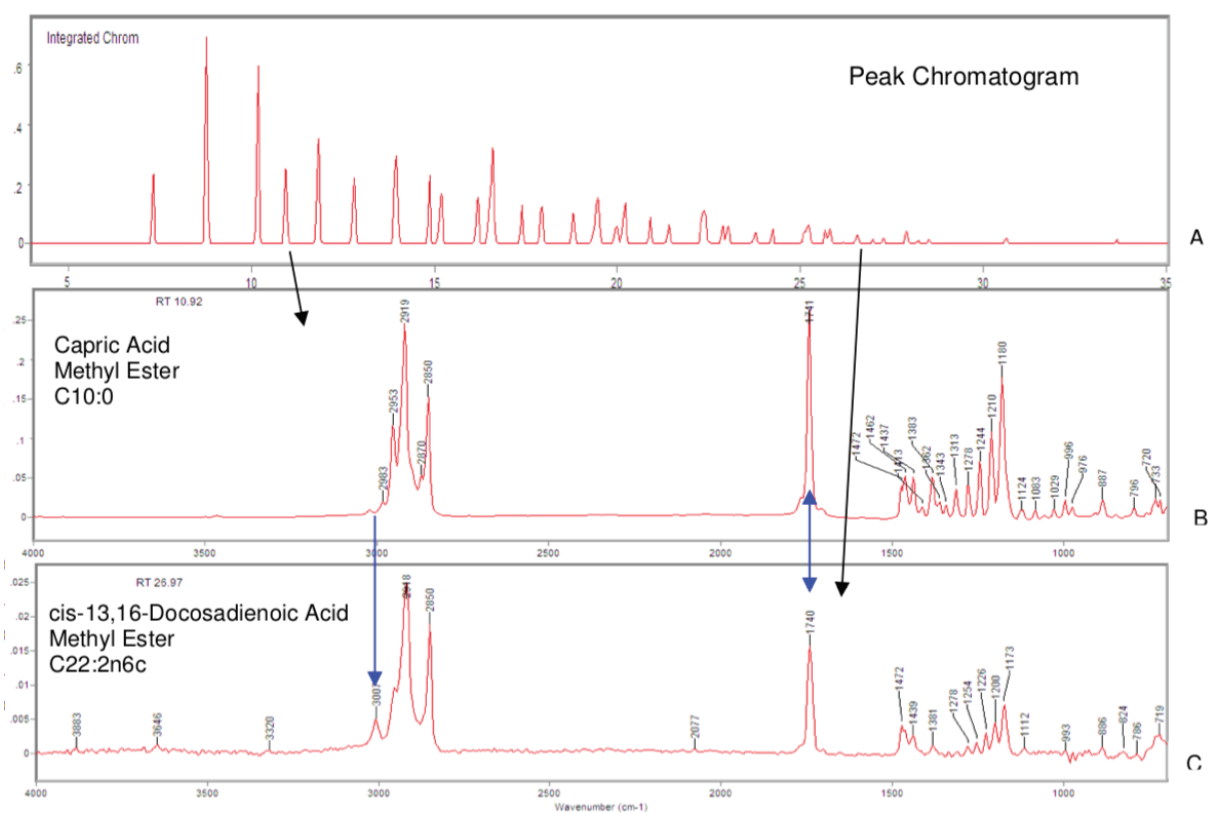


Fig. 2 IR Spectra for Saturated and Polyunsaturated Fatty Acid Methyl Esters from the same chromatographic run.

Panel A again shows the peak chromatogram. The full spectrum for each peak is collected in realtime and can be viewed and compared. The deposited sample remains available on the disc for re-analysis if desired.

Panel B represents the spectrum for Capric Methyl Ester, a saturated ten-carbon fatty acid eluting at 10.9 minutes in this separation.

Panel C is the spectrum for Docosadienoic Acid Methyl Ester, a twenty-two carbon fatty acid with two cis double bonds eluting at 26.9 minutes under these conditions. The appearance of the olefinic peak just above 3000 and the downward shift of the carbonyl near 1740 are hallmarks of higher saturation level. Comparison of unknown spectra to FAME standards can give information about chain length and saturation level, even without retention time data.

Spectral data of this type is particularly useful for fatty acids with unusual structures or those for which standards are not commercially available.

Selectivity of Infrared Spectroscopy in Analysis of *trans* Fats

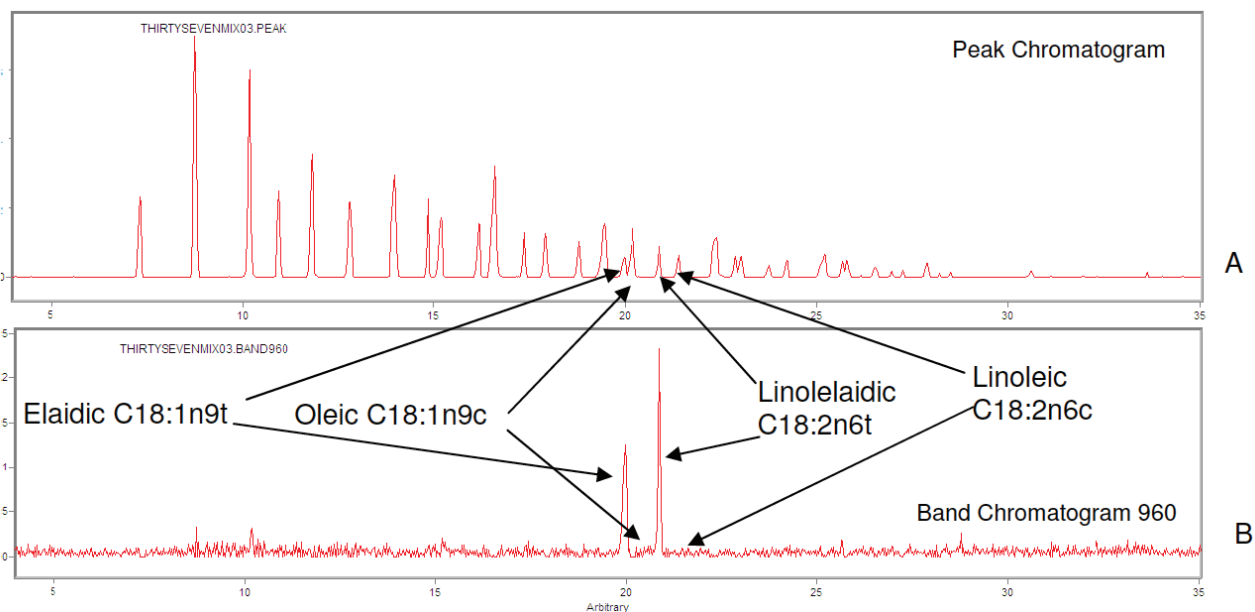


Fig. 3 Comparison of Peak Chromatogram to Band Chromatogram centered on 960cm⁻¹ of FAME mixture.

Panel A displays the peak chromatogram for the 37-component mixture.

Panel B shows the Band Chromatogram centered on 960 wavenumbers. In this plot, only the absorbance found in a defined range of the spectrum is plotted. This can be extremely useful when trying to discriminate between compounds on the basis of a particular structural feature. In this case, *trans* fats exhibit absorbance in a distinctive area between 955 -970 wavenumbers. As can be seen in this example, Elaidic and Linolelaidic Acids, with one and two *trans* double bonds respectively, show strong relative absorbance in this region compared to the neighboring peaks with only *cis* configuration. This alerts the analyst to examine the full spectra of these peaks to confirm that they are consistent with a *trans* configuration.

Table 1 Experimental Conditions:

Sample:	Supelco #47885 37 component Fatty Acid Methyl Ester Mixture
Injection	0.3 μ L using split/splitless injector, splitless:1
Column:	Supelco SP-2340-24022, 30m x 0.25-mm [100% poly(bis-cyanopropyl siloxane)]
Conditions:	Helium carrier, 1 mL/min
	Temp program: 40° for 1 min, 0°/min up to 140°C
	140° for 1 min, 4°/min up to 230°C
Injector, transfer line, restrictor tip:	239°C
Sample window	-50°C

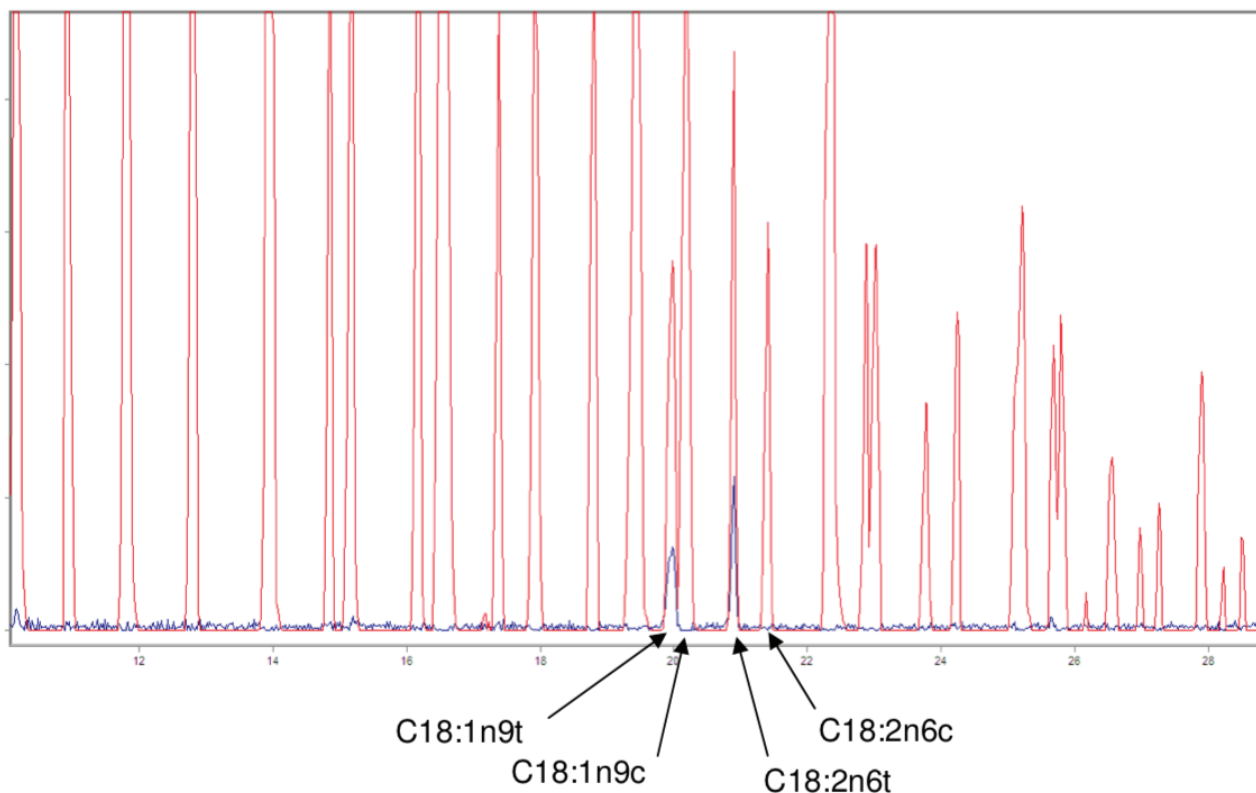
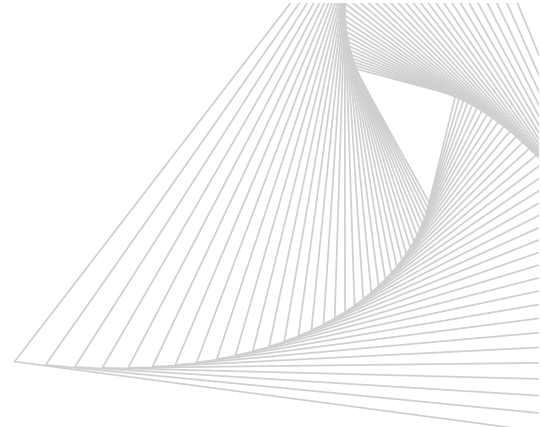


Fig. 4 Peak chromatogram and Band chromatogram at 960 cm^{-1} overlaid to illustrate the difference in scale between them due to the lower absorbance of the 960 band relative to that of other functionalities.

Table 2 FAME mixture components and relative concentrations:

1. Butyric Acid Methyl Ester (C4:0) 4%	15. <i>cis</i> -10-Heptadecenoic Acid Methyl Ester (C17:1) 2%	26. <i>cis</i> -11,14,17-Eicosadienoic Acid Methyl Ester (C20:2) 2% <i>r</i>
2. Caproic Acid Methyl Ester (C6:0) 4%	16. Stearic Acid Methyl Ester (C18:0) 4%	27. Behenic Acid Methyl Ester (C22:0) 4%
3. Caprylic Acid Methyl Ester (C8:0) 4%	17. Elaidic Acid Methyl Ester (C18:1 <i>n</i> 9 <i>t</i>) 2%	28. <i>cis</i> -8,11,14-Eicosatrienoic Acid Methyl Ester (C20:3 <i>n</i> 6) 2%
4. Capric Acid Methyl Ester (C10:0) 4%	18. Oleic Acid Methyl Ester (C18:1 <i>n</i> 9 <i>c</i>) 4%	29. Erucic Acid Methyl Ester (C22:1 <i>n</i> 9) 2%
5. Undecanoic Acid Methyl Ester (C11:0) 2%	19. Linolelaidic Acid Methyl Ester (C18:2 <i>n</i> 6 <i>t</i>) 2%	30. <i>cis</i> -11,14,17-Eicosatrienoic Acid Methyl Ester (C20:3 <i>n</i> 3) 2%
6. Lauric Acid Methyl Ester (C12:0) 4%	20. Linoleic Acid Methyl Ester (C18:2 <i>n</i> 6 <i>c</i>) 2%	31. Arachidonic Acid Methyl Ester (C20:4 <i>n</i> 6) 2%
7. Tridecanoic Acid Methyl Ester (C13:0) 2%	21. Arachidic Acid Methyl Ester (C20:0) 4%	32. Tricosanoic Acid Methyl Ester (C23:0) 2%
8. Myristic Acid Methyl Ester (C14:0) 4%	22. <i>g</i> -Linolenic Acid Methyl Ester (C18:3 <i>n</i> 6) 2%	33. <i>cis</i> -13,16-Docosadienoic Acid Methyl Ester (C22:2) 2%
9. Myristoleic Acid Methyl Ester (C14:1) 2%	23. <i>cis</i> -11-Eicoenoic Acid Methyl Ester (C20:1) 2%	34. Lignoceric Acid Methyl Ester (c24:0) 4%
10. Pentadecanoic Acid Methyl Ester (C15:0) 2%	24. Linolenic Acid Methyl Ester (C18:3 <i>n</i> 3) 2%	35. <i>cis</i> -5,8, 11,14,17-Eicosapentaenoic Acid Methyl Ester (c20:5 <i>n</i> 3) 2%
11. <i>cis</i> -10-Pentadecenoic Acid Methyl Ester (C15:1) 2%	25. Heneicosanoic Acid Methyl Ester (C21:0) 4%	36. Nervonic Acid Methyl Ester (C24:1) 2%
12. Palmitic Acid Methyl Ester (C16:0) 6%		37. <i>cis</i> -4,7,10,13,16,19-Docosahexaenoic Acid Methyl Ester (C22:6 <i>n</i> 3)
13. Palmitoleic Acid Methyl Ester (C16:1) 2%		
14. Heptadecanoic Acid Methyl Ester (C17:0) 2%		