Waters Integrity System Applications

Detection and Characterization of Triglycerides by ThermaBeam™ LC/MS

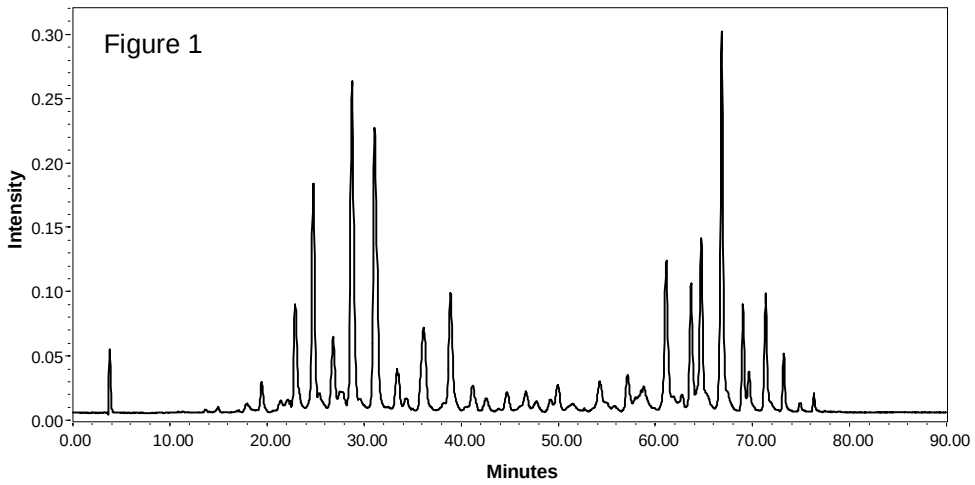
Highlights: Previously, triglycerides have been challenging to analyze by HPLC. Difficulties with typical LC detector technologies have led to the use of ThermaBeam LC/MS as a detection and identification alternative for triglycerides.

Triglycerides are known to be difficult compounds to analyze by HPLC using traditional detectors. These materials have absorbance maxima at low wavelengths, and, coupled with the high background absorbance of commonly used solvents, the applicability of UV detectors for triglygeride analysis is limited. Since gradient elution is commonly used, RI detectors cannot be utilized. The evaporative light scattering (ELS) detector, sometimes called, confusingly, a "mass detector," is an accepted detector for the HPLC analysis of triglycerides. Good baselines and chromatographic separation can be obtained using an ELS. However, no identification of the peaks in a separation can be obtained from this type of detector. The Waters Integrity LC/MS System features a ThermaBeam interface and Electron Ionization (EI) which offers the chromatographer mass detection and positive compound identification of chromatographic peaks. The unique ThermaBeam design adds a modest amount of heat to a pneumatic nebulizer to assist the aerosol formation process (50 degrees is adequate for triglycerides). The solvent evaporates in the heated expansion region, leaving solid particles or oil droplets depending on the sample. Note that up to this point, this parallels the mechanism for ELS operation. Subsequently, though, the particles/droplets formed during ThermaBeam nebulization pass through a two-stage momentum separator, each with higher vacuum, until they enter the ion source of the mass spectrometer.

Of the currently popular LC/MS interfaces, only ThermaBeam-like interfaces permit a sufficiently high vacuum in the ion source to allow electron impact (EI) ionization. EI is the accepted standard for producing mass spectra for qualitative interpretation and positive compound identification. This study describes how EI spectra can be used to determine the fatty acid composition of triglycerides.

Total Ion Chromatogram of Butterfat

Figure 1 represents a butterfat sample analyzed by the Waters Integrity System. This is the total ion current chromatogram obtained from the ThermaBeam Mass Detector.
The general appearance of the chromatogram is similar to that obtained with an ELS detector. Here, though, the EI mass spectra from each peak can be used to deduce the qualitative makeup of the triglycerides.

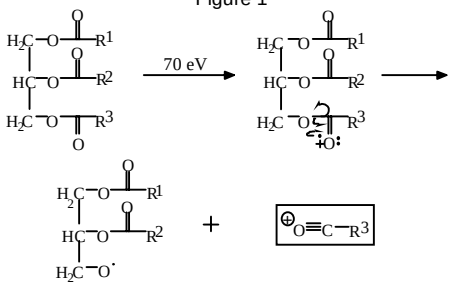


Chromatography Conditions

- ►Waters Symmetry™ C18 Columns, 3 x 150 mm.
  - ►Two columns to maximize resolution.
- ►Non-Aqueous Reversed Phase Elution.
  - ►Acetonitrile/Acetone mobile phase.
  - ►Flow 0.4 mL/min.
  - ►Pressure ca. 1100 psi. through interface.

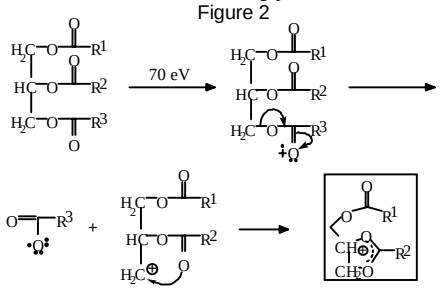
Formation of Acyl Ion

Figure 1



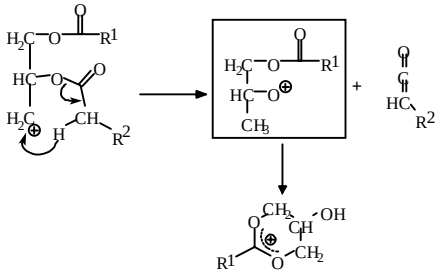
Formation of Diglyceride Ion

Figure 2



Formation of Monoglyceride Ion

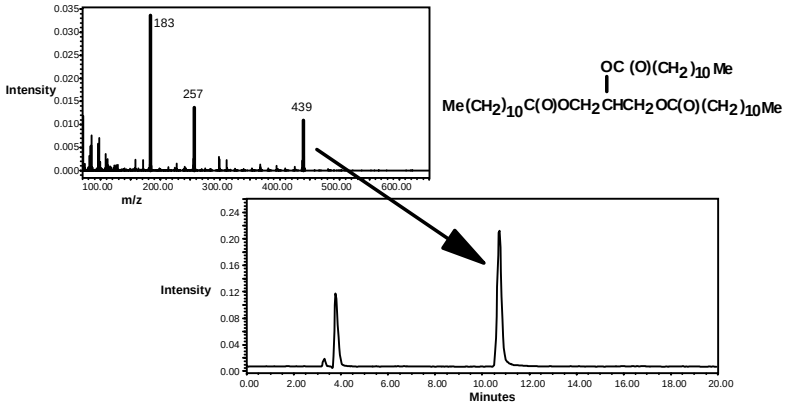
Figure 3



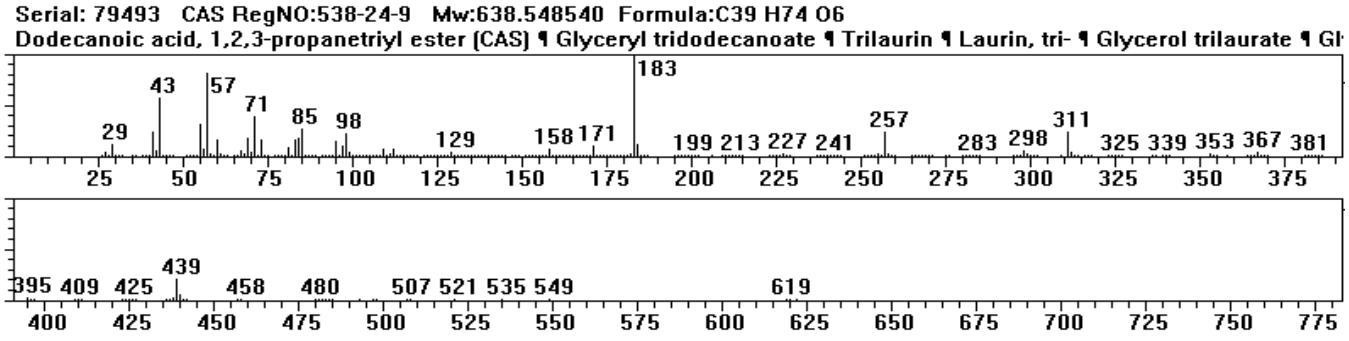
Electron ionization spectra are characterized by fragment ions derived from the molecular ion. The presence of certain fragment ions and their relative abundances form a unique "fingerprint" which is used to identify a compound of interest either by library matching or by spectral interpretation. Three classes of fragment ions are used to characterize triglycerides. The first, the acyl ions, are formed by homolytic cleavage of the molecular ion as shown in Figure 1. An acyl ion will be formed for each of the fatty acids present in the triglyceride. Tables of expected masses for these (and the other) ions can be prepared for ease in interpretation. Fragmentation of the molecular ion by heterolytic cleavage results in loss of an acyl chain as a neutral radical and a "diglyceride" ion as shown in Figure 2. There will be a diglyceride ion of a given mass value corresponding to each different fatty acid lost in fragmentation, i.e. 1,2, or 3 diglyceride ions for 1, 2, or 3 different fatty acids. Finally, Figure 3 illustrates the mechanism by which diglyceride ions can lose a second acyl group as a neutral ketene with resulting rearrangement of a hydrogen. There will also be one resulting "monoglyceride" ion for each fatty acid in the triglyceride.

Trilaurin

The data at right is from the analysis a simple triglyceride, trilaurin. There is a single peak in the chromatogram after the solvent peak. The EI mass spectrum shows acyl (m/z 183) and monoglyceride (m/z 257) ions at correct values for lauric acid. The diglyceride ion at m/z 439 has the correct mass for two retained lauric acid moieties.



Below is the Wiley library standard spectrum of trilaurin. Although the mass range for this library acquisition is much broader (m/z 5 to 780) the ions and their respective intensities in the overlapping mass range is almost identical. This is due to the "classical", reproducible nature of EI spectra.



These examples demonstrate how the chromatographer can utilize ThermaBeam EI with the Waters Integrity System to characterize and identify triglycerides based on mass spec interpretation and library search results.