Waters® Alliance® LC/MS System

Separation and Identification of Triacylglycerols of Peanut Oil by APcI LC/MS

Craig A. Dorschel, Waters Corporation, Milford, MA USA

Analytical Conditions

Columns: 2 Waters Symmetry® C_{18}, 3 x 150 mm

Mobile Phase: Acetonitrile/Acetone Gradient

Key Words
Triacylglycerols
Unsaturated fatty acids
APcI
Peanut oil

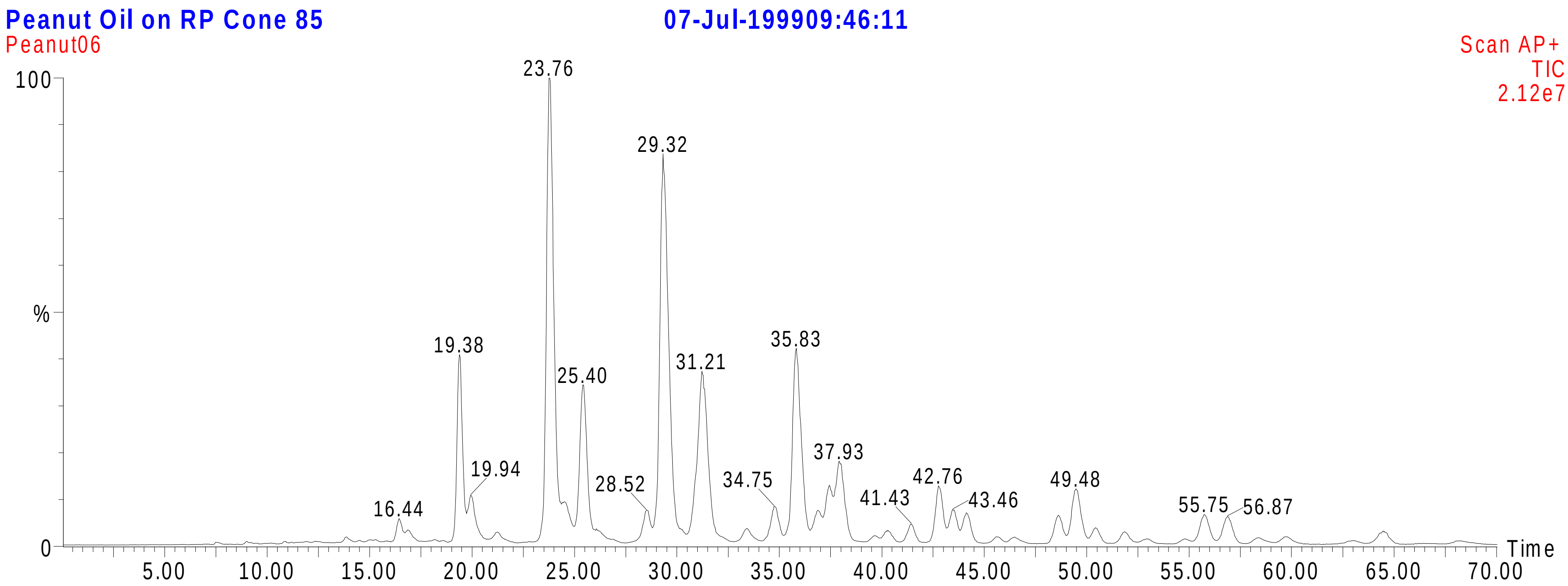
Atmospheric pressure chemical ionization (APcI) is well-suited for the LC-MS analysis of fats and oils. Using in-source collision-induced dissociation, the resulting fragments may be used to identify the fatty acid composition of the constituent triacylglycerols (TAGs).

This work was performed on a Micromass® Quattro LC mass spectrometer operated in single MS mode. Identical results can be expected from the Waters/Micromass ZMD.

Vegetable oils, such as peanut oils, are complex mixtures composed primarily of triacylglycerols. Separation of the components is achieved by chromatography on reversed phase solid phases using organic solvents as eluents. TAGs elute primarily in order of increasing equivalent carbon number, the total number of carbons in the constituent fatty acids of a TAG less two for each olefinic double bond in the fatty acids. For a given equivalent carbon number, elution times are affected by the degree of unsaturation, with the most unsaturated TAGs eluting first.

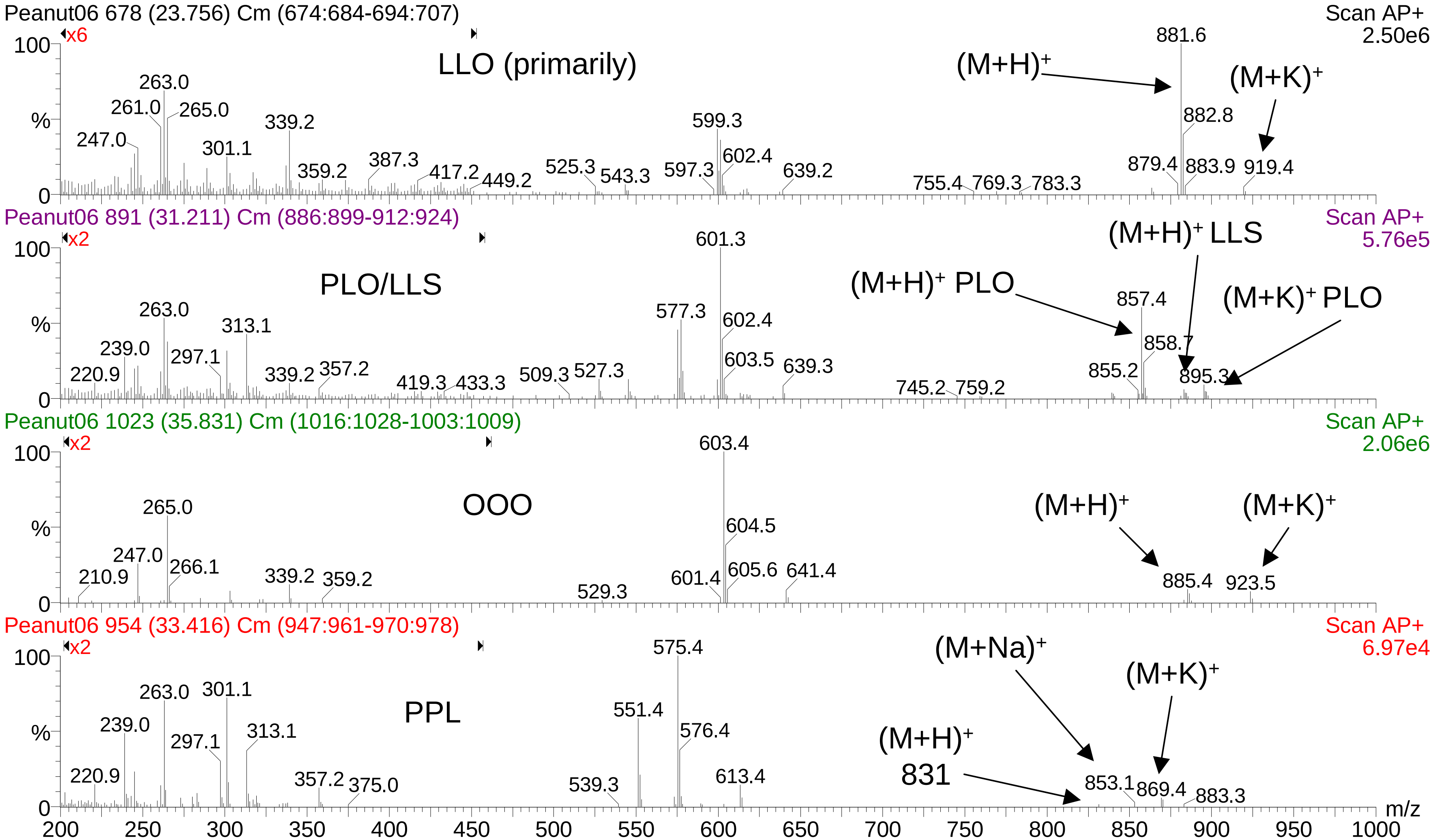
Use of a mass spectrometer as the detector component allows identification of the fatty acids comprising a TAG in addition to providing the chromatographic profile of the sample.

Because of the non-polar nature of TAGs, atmospheric pressure chemical ionization (APcI) is preferred to electrospray as the method of choice for atmospheric pressure ionization mass spectrometry. The total ion current chromatogram for peanut oil is shown below:



Identification of the fatty acids of a TAG is achieved by observation of characteristic fragment ions in the mass spectrum, along with the pseudomolecular ions $(M+H)^+$. The fragment ions are of three types: acyl ions $(RCO)^+$, monoglyceride ions $[(M+H)\text{-}RCOO\text{-}R'CO]^+$, and diglyceride ions $[(M+H)\text{-}RCOOH]^+$, where R and R’ represent the carbon chains of fatty acids. There will theoretically be an acyl and a monoglyceride ion for each different fatty acid present, although not all such ions are observed in APcI. Additionally there will be a diglyceride ion for each possible pair of fatty acids in a particular TAG. Spectra for representative examples from this chromatogram follow:

Peanut Oil on RP Cone 85

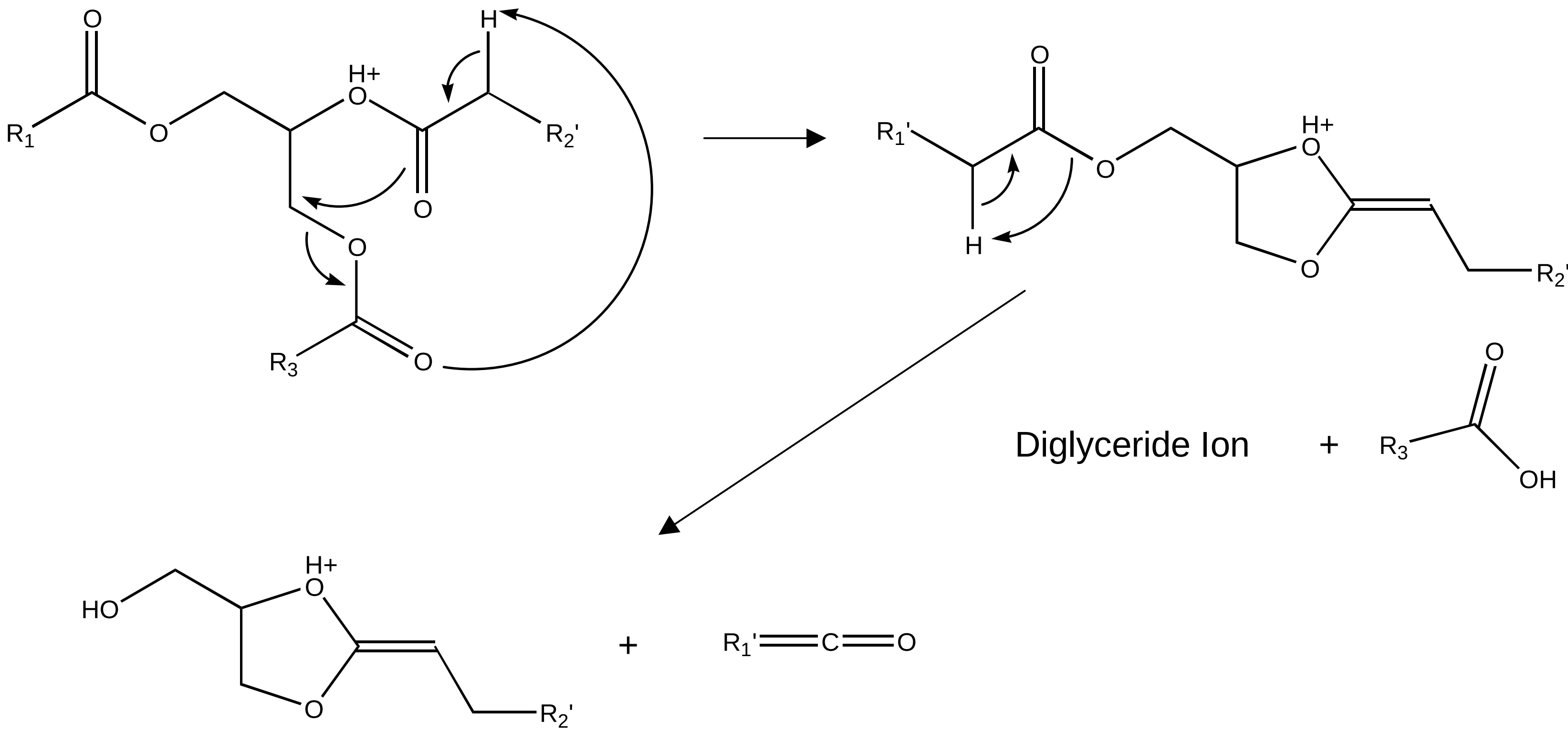


The intensity of the $(M+H)^+$ ion varies considerably. In the examples shown, it is the base peak for the top spectrum (LLO), where there are two polyunsaturated fatty acids. As saturated and monounsaturated fatty acids are substituted, the intensity of $(M+H)^+$ diminishes to the point where it is barely visible in the bottom spectrum. Alkali metal ion complexes (with sodium and especially potassium) are common and should not be confused with $(M+H)^+$. Potassium ion adducts are also seen in the acyl $[(RCO-H+K)^+]$ and diglyceride $\{[(M+K)-RCOOH]^+\}$ series.

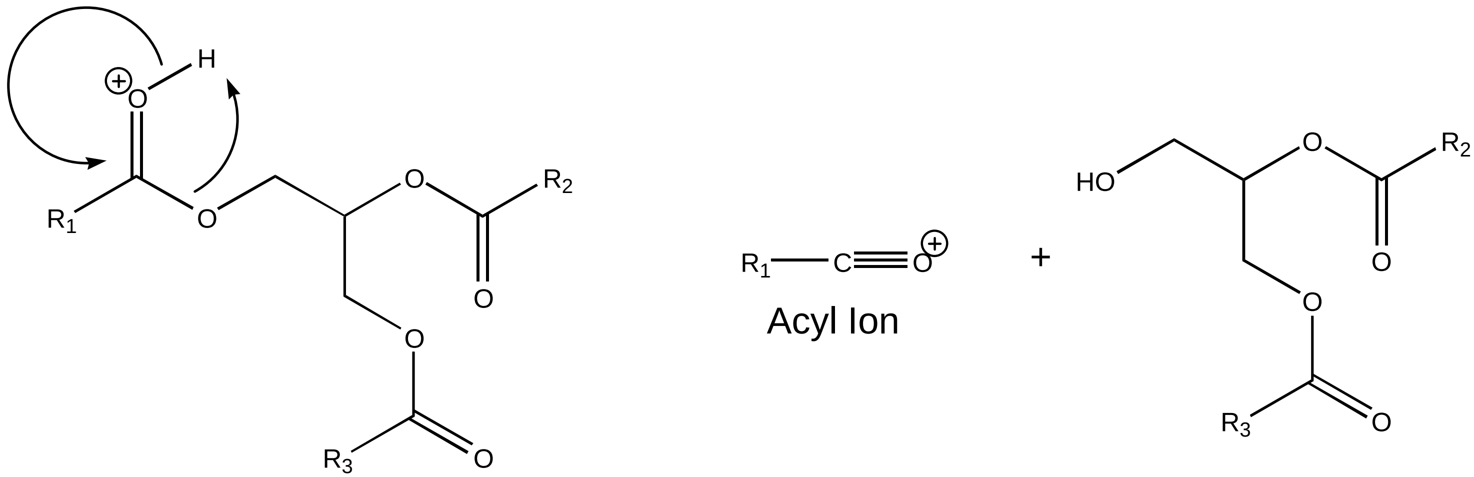
Fragmentation of the $(M+H)^+$ ions to diglyceride ions occurs via collision-induced dissociation irrespective of cone voltage setting. Increasing the cone voltage to *ca.* 85 volts enhances fragmentation to the acyl and monoglyceride ions useful for identifying fatty acids. The fragmentation may be rationalized as illustrated by the scheme below. The fatty acid composition of a TAG is determined by first cataloging the fatty acids present using acyl and/or monoglyceride ions and then observing the diglyceride ions to determine which combinations are present. The proposed composition should correspond to the observed $(M+H)^+$. The expected mass value for $(M+H)^+$ is simply obtained by adding 90 to the sum of the masses of the three acyl ions for the proposed composition.

The actual composition of peanut oil is quite complex. In addition to TAGs containing palmitic, stearic, oleic, linoleic, and linolenic acids common to vegetable oils, TAGs containing arachidic ($C_{20:0}$), gadoleic ($C_{20:1}$), behenic ($C_{22:0}$), lignoceric ($C_{24:0}$), and cerotic ($C_{26:0}$) acids were identified.

Fragmentation Scheme



Monoglyceride Ion


Acyl Ion