

# Lipid Analysis by Reversed-Phase HPLC and Corona CAD: Triglycerides

Triglycerides (TGs) are formed from a single molecule of glycerol, combined with three fatty acid side chains. The fatty acids are normally of an even number of carbon atoms, typically from 16 to 20, and may either be fully saturated or partially unsaturated. Consequently, a tremendous variety of TGs can be found *in vivo*. These molecules play an important role in metabolism, acting as an energy store and a reservoir of free fatty acids.

The Corona® charged aerosol detector (CAD®), a sensitive mass-based detector, is especially well-suited for the determination of lipids such as TGs. When combined with reversed-phase chromatography, this detector enables the quantitation of low level analytes covering a wide range of molecules, from free fatty acids to pure alkanes, while maintaining sufficient specificity to resolve impurities down to low-nanogram levels.

This application note illustrates the ability of reversed-phase HPLC with Corona CAD detection to resolve and measure numerous closely-related TGs in various natural oils. In comparison to the earlier work of Holcapek *et al.*, (Quantitation of triacylglycerols in plant oils using HPLC with APCI-MS, evaporative light-scattering, and UV detection. (2005), *J. Sep. Sci.*, **28**, 1315-1333), the RP-HPLC Corona CAD approach is capable of resolving many more TGs in natural oils.

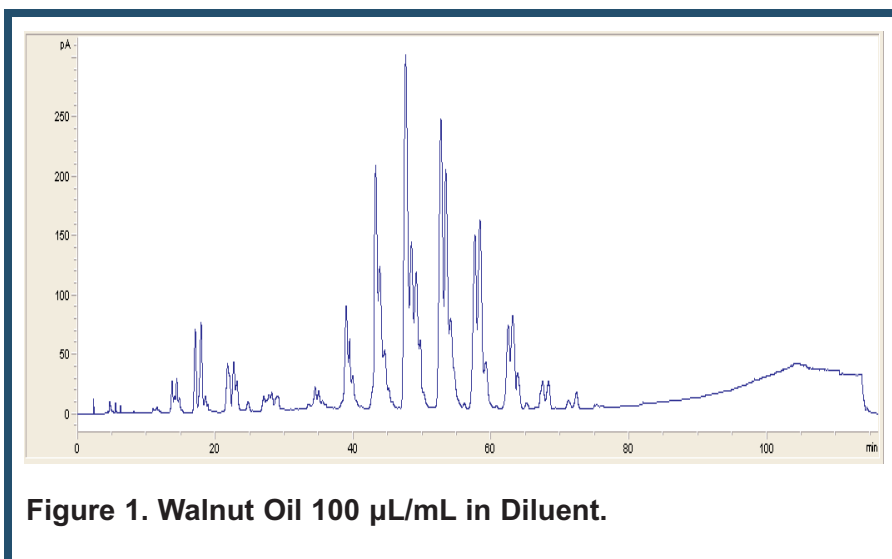


Figure 1. Walnut Oil 100 µL/mL in Diluent.

## Method Parameters

Column: \*Halo C8, 150 x 4.6 mm, 2.7 µm, 40°C  
 Detector: Corona CAD Plus  
 Nebulizer Heater: On  
 Filter: None  
 Mobile Phase A: Acetonitrile  
 Mobile Phase B: *iso*-Propanol  
 Gradient Profile: Table 1  
 Flow Rate: 1.2 mL/min  
 Run Time: 115 minutes  
 Injection Volume: 10µL at 10°C  
 Diluent: Methanol/chloroform (1:1, v/v)

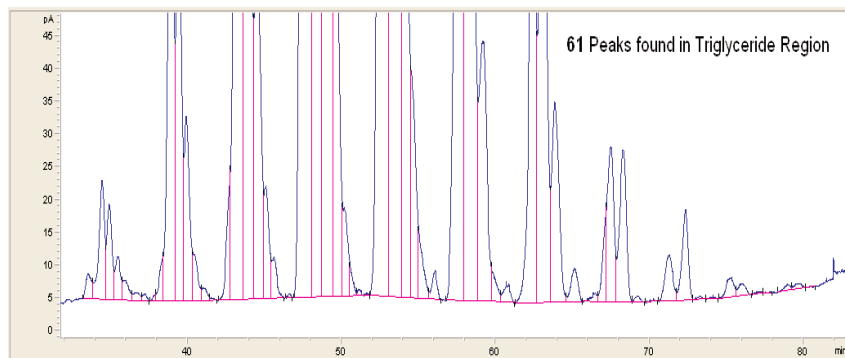
Time	%A	%B
0.0	100	0
100.0	20	80
100.0	20	80
110.1	100	0
115.0	100	0

Table 1. Gradient Profile.

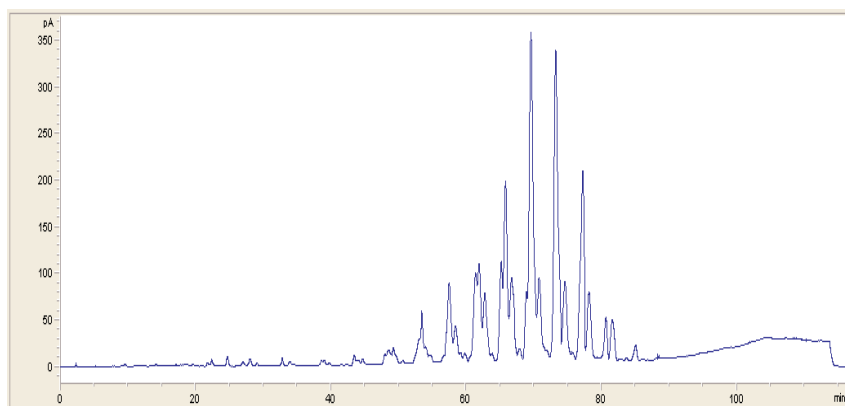
## Results and Discussion

A complete chromatogram of walnut oil is shown in Figure 1, and an integrated chromatogram of the different triglycerides is presented in Figure 2. In total, the Halo column was capable of resolving sixty-one peaks in the TG region, in ~90 mins. Using two C18 columns in series, and UV (205 nm), ELSD and ACPI-MS detection, Holcapek *et al.*, (2005) identified 43 TGs in ~106 mins. However, these authors were able to identify many of the different TGs present in their oil sample.

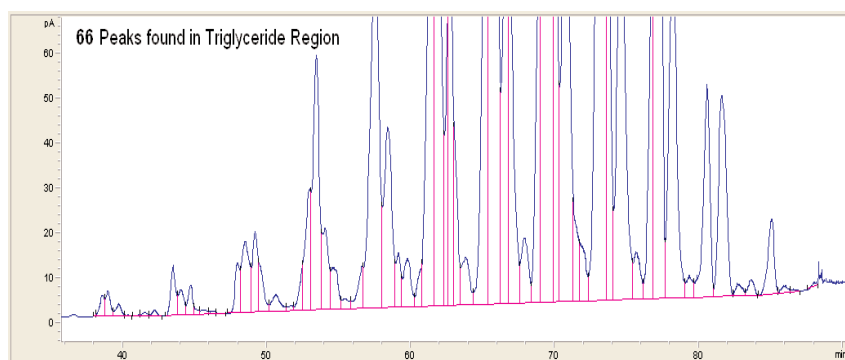
Mustard oil, an oil common in Mediterranean and the Bengali region of India, finds uses as an aromatic cooking oil and as therapeutic ointment for stiff joints and muscles. The oil is high in the healthy omega-3 fatty acids without high levels of the non-beneficial omega-6 fatty acids found in other oils. A complete chromatogram of mustard oil is shown in Figure 3. Figure 4 shows the expanded and integrated TG region with a total of 66 peaks.



**Figure 2. Walnut Oil 100  $\mu$ L/mL in Diluent: Expanded Triglyceride Region.**

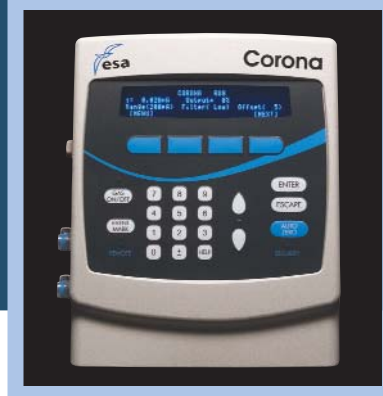


**Figure 3. Mustard Oil 100  $\mu$ L/mL in Diluent.**



**Figure 4. Mustard Oil 100  $\mu$ L/mL in Diluent: Expanded Triglyceride Region.**

# The Corona<sup>®</sup> Charged Aerosol Detector



## Conclusions

The general method described in this application note is capable of separating most of the lipid classes in natural oils. The method can be used for the characterization and quantitation of different lipid species in a variety of natural oils samples. This method can also be used to generate a "lipid fingerprint" for many kinds of samples and is of use to those studying the lipidome. By simple modification, it can be tailored for a specific lipid class in order to decrease analytical run time.

## Ordering Information

Corona Plus Charged Aerosol Detector	70-7041
Thermal Organizer Module	70-5499TA
Nitrogen generator	70-6003
Pump, model 584**	70-7091
Quaternary Low Pressure Gradient	70-5260
Autosampler, model 542	70-4152
EZChrom Elite <sup>™</sup> for ESA software including PC	70-5073

\*ESA wishes to thank Mac-Mod Analytical, Inc. (Chadds Ford, PA) for the use of their column.

\*\*Modification required - contact ESA Technical Support



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