

Determination of Polymerized Triglycerides by High Pressure Liquid Chromatography and Corona Veo Charged Aerosol Detector

*Qi Zhang, Bruce Bailey, Dave Thomas, Marc Plante, and Ian Acworth
Thermo Fisher Scientific, Chelmsford, MA, USA*



Overview

Purpose: To create a reverse phase high pressure liquid chromatography (HPLC) method to determine polymerized triacylglycerides in used cooking oil, using a single calibrant, as a demonstration of the universality of charged aerosol detection with non-volatile analytes.

Methods: A ternary gradient, HPLC method using a non-porous C18 column was developed to separate and quantify polymeric triacylglycerides as a sharp peak and without a standard.

Results: The method was quantitative for both the triacylglycerides and the polymerized triacylglycerides using a single, linear calibration standard.

Introduction

Triacylglycerides (TAGs), which form the bulk of vegetable and animal oils, are subject to polymerization reactions when heated. These reactions occur by thermal polymerization¹ or by oxidative polymerization reaction via radicals. These polymeric compounds create high-boiling point, higher viscosity,^{2,3} and insoluble materials, which can affect product quality and properties. For cooking, these polymerized triglycerides (PTGs) can result in increased oil absorption into cooked foods,⁴ gumming of the fry bath, and possibly unhealthful conditions such as reduced digestibility.⁵

The current method for the determination of these PTGs is by HPLC with size exclusion chromatography (SEC) and refractive index (RI) detection, which also requires a solid phase extraction (SPE) process to remove these polymers from the TAGs.⁶ A reverse phase HPLC method was developed, using a C18 column and gradient elution chromatography using the Thermo Scientific™ Dionex™ Corona™ Veo™ Charged Aerosol Detector. This method has several advantages: no SPE is required, as the TAGs do not interfere with the analysis of the PTGs, the chromatography provides more information in the equivalent time, and the detector is highly sensitive and precise, thereby improving data quality.

Charged aerosol detection (CAD) is a mass sensitive technique for determining levels of any non-volatile and many semi-volatile analytes after separation by HPLC. The use of CAD for the analysis of lipids is well documented, as it will detect any non-volatile analyte with a uniform response factor and allows use of gradient methods, unlike refractive index detectors. HPLC methods using Corona Veo charged aerosol detection have limits of detection typically between high-picograms to low nanograms on column and have a wide dynamic range from nanogram to microgram levels, with high reproducibility. A schematic of the Corona Veo charged aerosol detector is shown in Figure 1. Unlike ELSD, no changes in gain settings are needed to determine high and low-level analyte concentrations.

For non-volatile analytes (greater than approximately 350 °C standard pressure boiling point), the Corona detectors can provide a uniform response factor. This allows the use of a single calibrant to determine the amounts of other, non-volatile analytes. The TAGs and polymers are both non-volatile, and a calibration plot created for TAGs was found to be valid for the determination of the amounts of polymer present in heated oil samples.

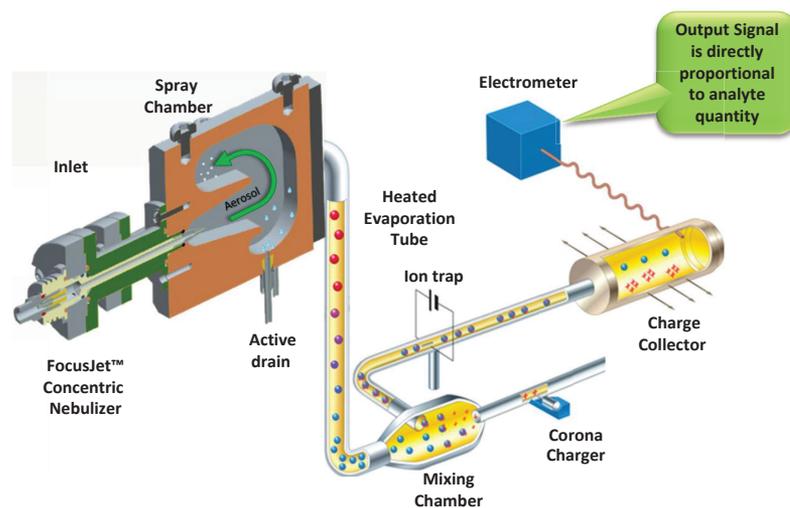


FIGURE 1. Schematic of the Corona Veo Charged Aerosol Detector.

Methods

Sample Preparation

The standard used, extra virgin olive oil (EVOO), was dissolved in methanol/tetrahydrofuran (THF) (1:1) at an initial concentration of 10.0 mg/mL, and diluted incrementally by 50% to a low concentration of 78 ug/mL. The first standard used in the calibration was at a concentration of 5.00 mg/mL.

Heated oil samples were prepared by heating 200 mL of EVOO in a 1 L, covered beaker to a temperature of 270 °C. Aliquots of oil were removed with a metal spatula and stored in HPLC vials for later dilution.

Samples, including refined safflower oil, were dissolved in methanol/THF (1:1) at a concentration of 10.0 mg/mL and then diluted 50%. Samples with undissolved material (samples heated for 240, 270, and 300 minutes) required the addition of THF first, and then dilution with an equivalent volume of methanol.

Liquid Chromatography

HPLC System: Thermo Scientific™ Dionex™ UltiMate™ 3000 system consisting of a DGP-3600RS pump, WPS-3000TRS autosampler, and TCC-3000RS column oven

Mobile Phase A: Water/methanol/acetic acid (600:400:4)

Mobile Phase B: Acetonitrile/acetic acid (1000:4)

Mobile Phase C: 2-Propanol/THF (800:200)

Sample Temp.: 10 °C

Injection Volume: 2.00 µL

Column: Imtakt Presto-FF* C18, 2 µm, 4.6x150 mm

Column Temp.: 40 °C

Detector: Corona Veo RS

Evaporation Temp.: 80 °C

Data Rate: 10 Hz

Power Functions: 0 – 19 minutes, 1.3; 19 – 27 minutes, 2.0

Filter: 5 seconds

Gradient:

Time (minutes)	Flow Rate (mL/min)	%A	%B	%C
-6	0.4	98	2	0
0	0.4	98	2	0
4	0.4	72	28	0
15	0.9	0	100	0
17	1.0	0	100	0
18	0.6	0	0	100
21	0.5	0	0	100
22	0.5	0	100	0
25	0.5	0	100	0
26	0.5	98	2	0
27	0.6	98	2	0

Data Analysis

All data was collected and processed using the Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) 7.2 SR3 software and data station.

Results

Chromatography

A non-porous C18 column was selected to avoid polymeric analytes from accumulating into the pores of packing media and thereby impairing performance.

For aerosol-based detectors, and where water content changes significantly across a gradient, an inverse gradient is typically employed to eliminate changes in response factor of analytes across the gradient. In this method, the main analytes elute from the column at 100% organic eluent, no inverse gradients were required to maintain a consistent response factor for these non-volatile analytes.

In having the polymer peak as a single peak, the sensitivity and quantitation is simplified, as all of the polymers are eluted at the same / similar retention time, and only one power function value needs to be determined for this complex analyte.

Calibration

The standard used for calibration purposes was the EVOO, itself, which is comprised of TAGs with small amounts of diacylglycerides (DAGs), and monoacylglycerides (MAGs). In using standards with known concentrations, the total peak area can be used to calibrate the instrument for the response factor for these analytes. Since CAD detects analyte particles, with compound volatility as the major factor with regards to signal, "the Corona charged aerosol detector is characterized by a uniform response toward aerosol-forming compounds with low vapor pressures," or high-boiling points.⁷

Calibration linearity is an important factor for a quantitative method. Since charged aerosol detection is inherently nonlinear, response factors can vary with peak height. Thus, differently shaped peak exhibit different response curves. By using the CAD power function feature, response variability for different peaks can be eliminated. The CAD power function can be used to provide linear peak area correlations with mass.

To determine the power function value for the TAGs, five concentrations of EVOO were injected and analyzed. A chromatogram of 5 μg on column (o.c.) of EVOO is shown in Figure 2. When a non-linear calibration plot is subjected to different Power Law values in Chromeleon CDS 7.2, a convenient way to determine the power function value (PFV) for CAD output is provided. A linear calibration fit can then be obtained when correct PFV values are used and for the TAGs a PFV of 1.30 provided the best, linear fit.

Since no standard or known concentration was available for the polymer peak, the sample obtained after 150 minute of heating was injected with four different injection volumes. Injection volumes of 1.0, 1.5, 2.0, and 2.5 μL were analyzed and response was plotted to create known response differences between injected amounts. This served as a means of calibration for the polymeric TAGs. Using Chromeleon Power Law, a PFV of 2.00 was determined. A chromatogram of EVOO, heated at 270 $^{\circ}\text{C}$ for 210 minutes is shown in Figure 3 and this illustrates the amount of polymeric TAGs produced.

After the power function values were determined, method parameters were adjusted using these values during specified retention time windows (see Method Conditions). A calibration sequence was performed, and the peak area vs. total glycerols was plotted and fit to a linear equation, with an $R^2=0.9997$, as shown in Figure 4. The corresponding, volume-calibration plot for the polymer peak is shown in Figure 5.

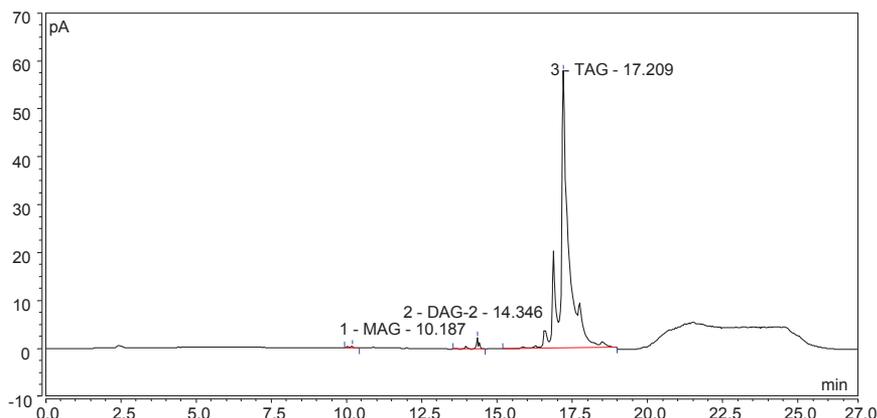


FIGURE 2. HPLC-CAD chromatogram of 5 μg o.c. of EVOO in methanol/tetrahydrofuran (1:1).

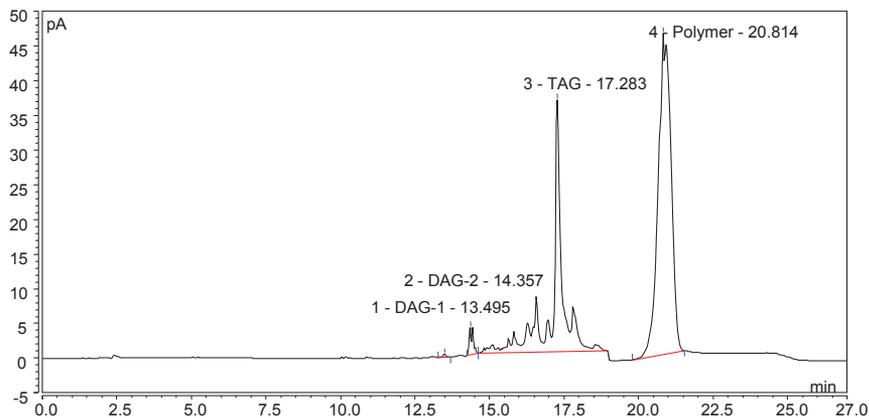


FIGURE 3. HPLC-CAD chromatogram of EVOO (heated for 210 minutes at 270 $^{\circ}\text{C}$) in methanol/tetrahydrofuran (1:1).

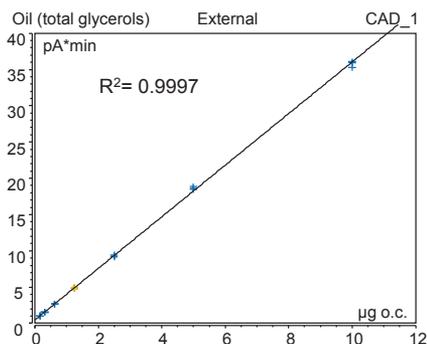


FIGURE 4. Linear calibration fit of total EVOO glycerols, from 0.156 – 10 µg o.c., each in triplicate, PFV = 1.30.

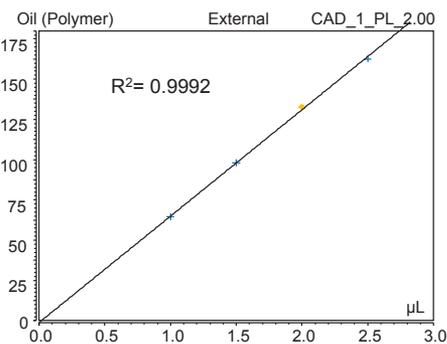


FIGURE 5. Power Law linear calibration fit of EVOO-polymers, with PFV = 2.00, using four, different injection volumes (y-axis not scaled).

Sample Analysis

The heated EVOO samples were analyzed and all of the acylglycerol peaks were integrated and quantified with the TG calibration equation. The polymer peak was also integrated and quantified using the same equation. The trend of percent-polymer in the heated oil samples was plotted over time and is shown in Figure 6.

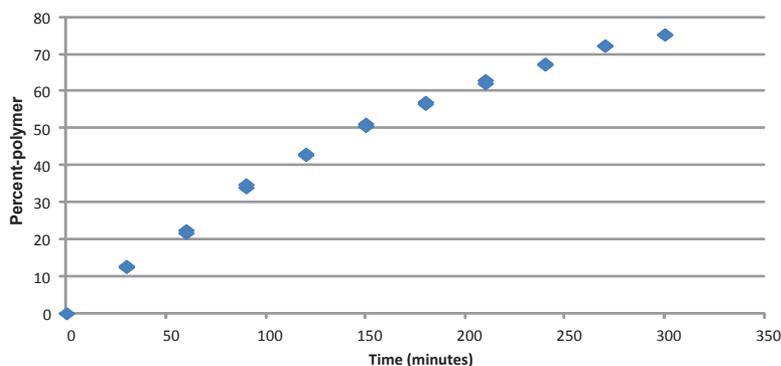


FIGURE 6. Percent polymer in 270 °C-heated EVOO over five hours' time, n=2.

Calibrations based on detector uniform response factors for non-volatile analytes are possible using the CAD. To verify that a valid quantitative method of analysis was developed, the total amounts of EVOO peaks (MAGs, DAGs, and TAGs) and the total polymer peak produced during heating periods (shown in Figure 6) were evaluated. Since both the EVOO peaks and the unknown and complex polymer peak are assumed to be non-volatile the calibration results used in this study should be valid.

To confirm the validity of this calibration process, the percent mass found (relative to 10 µg o.c.) was plotted over all injections, covering the complete range of percent-polymer produced, and is shown in Figure 7. The total range of absolute error across the experiment was approximately $\pm 3\%$.

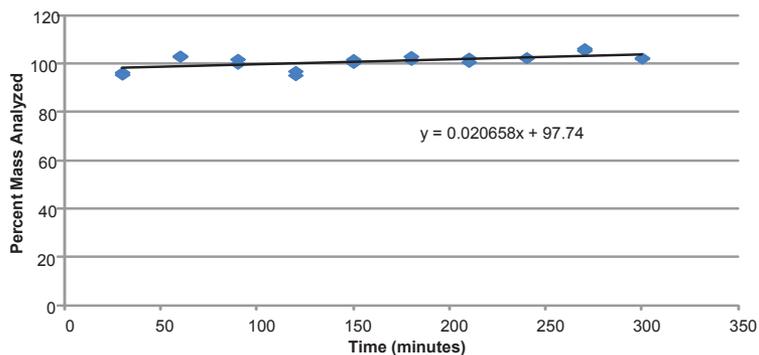


FIGURE 7. Percent total quantified mass over five hours' time, n=2.

A sample of refined safflower oil was analyzed, and a chromatogram is shown in Figure 8. Polymers can be created during the deodorizing and refining processes, and a small polymer peak is found with a relative mass of 0.12%.

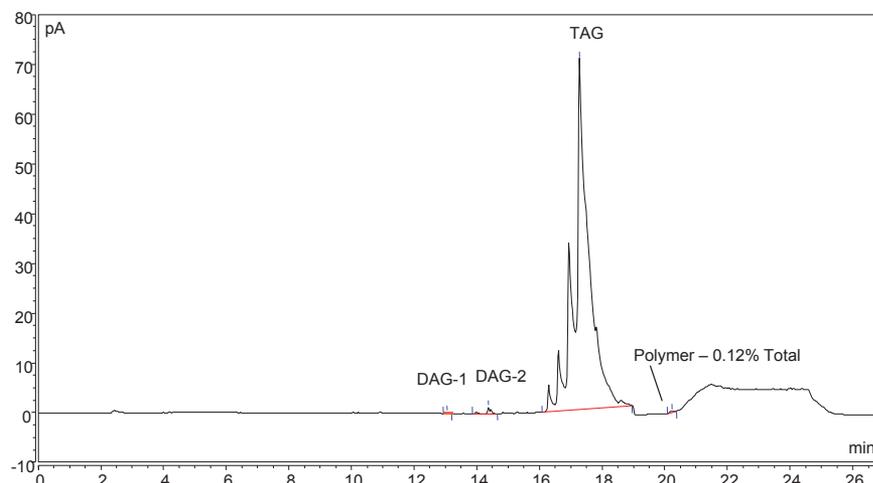


FIGURE 8. HPLC-CAD chromatogram of refined safflower oil.

Conclusions

- A method was created to determine polymeric triacylglycerides in vegetable oils, from low to high levels without the need of a polymer standard.
- The Corona Charged Aerosol Detectors can provide quantitative, analytical results for all non-volatile analytes (both simple and complex) when uniform response factors are produced.
- When non-volatile analytes are analyzed using linear calibration fits, it is possible to use calibrations obtained with one standard to determine amounts of all other non-volatile analytes since the detector provides uniform response factors.
- Water content changes when using gradient methods can alter detector response because nebulizer efficiencies change. This can be easily mitigated by using an inverse gradient so that uniform response factors can be obtained for all analytes. No inverse gradient was required for this method.

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