

Simultaneous Analysis of Glycerides (Mono-, Di-, and Triglycerides) and Free Fatty Acids in Palm Oil

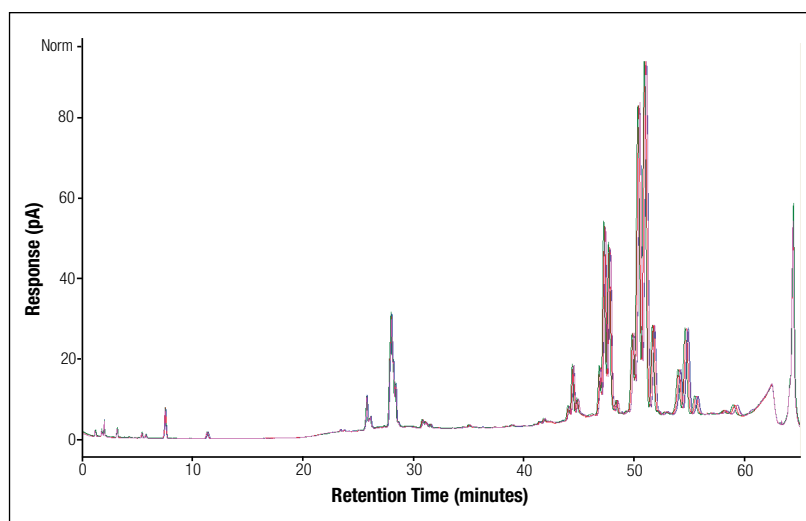


Figure 1. Replicate analysis of palm oil sample by HPLC-charged aerosol detector (n = 4; ~74 μ g on column).

Palm oil is the second largest edible oil produced in the world. It is comprised of mainly triglycerides with some mono and diglycerides. Free fatty acids and some other sediments and components in the crude oil are considered impurities and may be removed in the refining process. The level of refinement varies depending on source and final use.

Analytical methods for oils, like palm oil, often use gas chromatography/flame ionization detection (GC/FID) methods with derivatization. A high-performance liquid chromatography (HPLC) method was developed for the analysis of palm oil using a binary pump system with Thermo Scientific Dionex Corona Charged Aerosol Detector for detection. The method requires simple sample preparation and no derivatization. Comparisons were made to the Association of Analytical Chemists (AOAC) Official Method 993.24, which uses a differential refractometry detector. The repeatability and selectivity of the system was checked as well as the limit of detection (LOD) for individual components.

Method Parameters

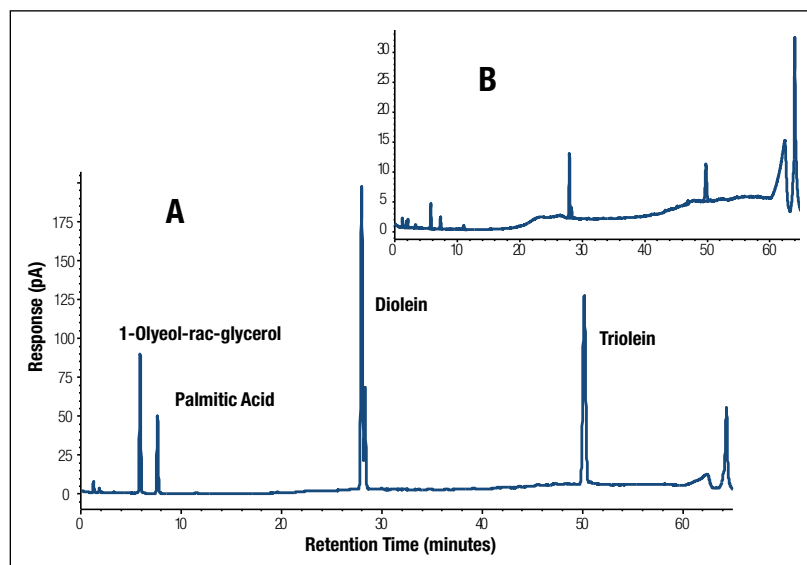
Column:	C18, 4.6 mm I.D. \times 250 mm, 5 μ m at 30 $^{\circ}$ C
Nebulizer Heater:	30 $^{\circ}$ C
Filter:	None
Mobile Phase A:	75% acetonitrile, 12% methanol, 8% HPLC-grade water, 4% THF, and 0.3% acetic acid
Mobile Phase B:	90% acetone and 10% acetonitrile
Gradient Profile:	Table 1
Flow Rate:	1.5–1.9 mL/min
Run Time:	70 min

Table 1. Gradient conditions

Time	%A	%B	Flow Rate
0.00	100	0	1.5
5.00	100	0	1.5
11.00	96	4	1.5
24.00	37	63	1.5
35.00	32	68	1.5
45.00	18	82	1.9
58.00	15	85	1.9
60.00	5	95	1.5
63.00	100	0	1.5
65.00	100	0	1.5

Table 2. The average peak area and relative percent deviation for four analyses of palm oil at ~74 μg (on column)

	Average Peak Area	%RSD
Total Triglycerides	6900	0.64
Peak at 50 Minutes	1478	0.92
Palmitic Acid	72	1.63

Figure 2A. Standard chromatogram (~2.5 μg on column). Figure 2B. Standard chromatogram (~95 ng on column).

Sample Preparation and Analysis

The material analyzed was a commercially available sample of red palm oil from Jungle Products (Bloomfield, USA). The oil is listed as being from the fruit of the *Dura* plant and in its natural state without refinement. The sample was simply dissolved and diluted in a 1:1 solution of methanol and tetrahydrofuran (THF). The concentration used for analysis was ~7.4 mg/mL. The palm oil sample was injected multiple times on multiple days to show reproducibility and robustness.

Standards of palmitic acid (P-0500, Sigma-Aldrich, St. Louis, USA), 1-oleoyl-rac-glycerol (M7765, Sigma-Aldrich), 1,3 diolein (D3627, Sigma-Aldrich), and triolein (T7140, Sigma-Aldrich) were also prepared in a 1:1 solution of methanol and THF. The standards were diluted and run at concentrations from 250 $\mu\text{g/mL}$ to 3 $\mu\text{g/mL}$. 10 μL injections were made throughout the analysis for all standards and samples. The data were processed and the response versus the column load was plotted.

Results and Discussion

Reproducibility of the method was tested by comparing four different injections of the Palm oil sample at 74 μg column load. The peak areas for an individual peak as well as groups of peaks were observed and the results are shown in Table 2. The peak retention times exhibited less than 1% variation over the gradient run for all samples observed. Four overlays of the Palm oil are shown in Figure 1. The chromatograms for the four standards run are illustrated in Figures 2A and 2B. The correlation is done using a polynomial fit and was greater than >0.999 for all points (Figure 3). The LOD was <32 ng on column for the mono-, di-, and triglyceride standards. A matrix effect was observed at the retention time seen for Palmitic acid and interfered with the peak at low levels. The observed limit of quantification (LOQ) was therefore ~ 100 ng on column for the palmitic acid.

A correlation was performed with the data collected using the CADTM Charged Aerosol Detector (Figure 4A) and the data discussed in the AOAC method (Method #993.24: Triglycerides (by Partition Numbers) in Vegetable Oils Liquid Chromatography Method IUPAC-AOCS-AOAC Method). The elution order observed in this method correlated well with the Equivalent Carbon Number as discussed in the AOAC method. The percent contribution for each group (shown in Figure 4B) was calculated by adding the individual area together and then dividing this by the total triglyceride peak areas. The correlation between the values obtained in the single sample analyzed by Corona charged aerosol detector and the large study done by the AOAC are compared in Figure 5, and show a good correlation.

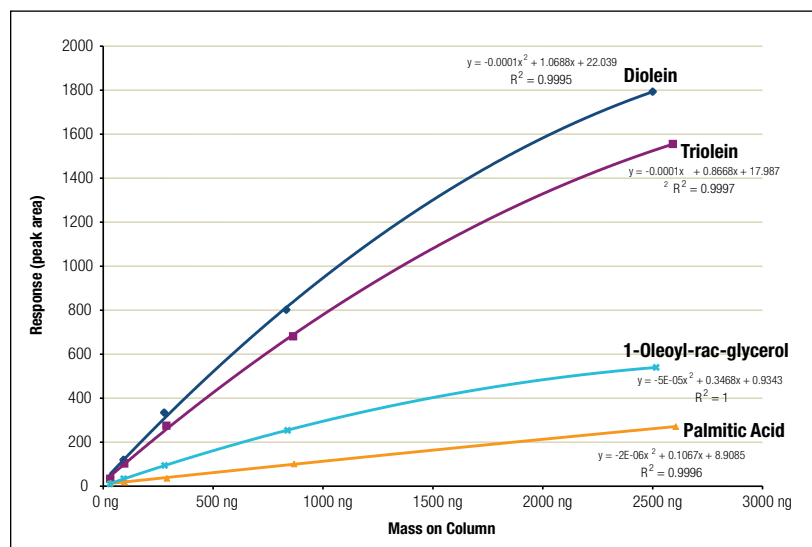


Figure 3. Standard calibration curves. The fit was achieved by using the polynomial fit function second order and the equations and correlation coefficients are displayed on the plot.

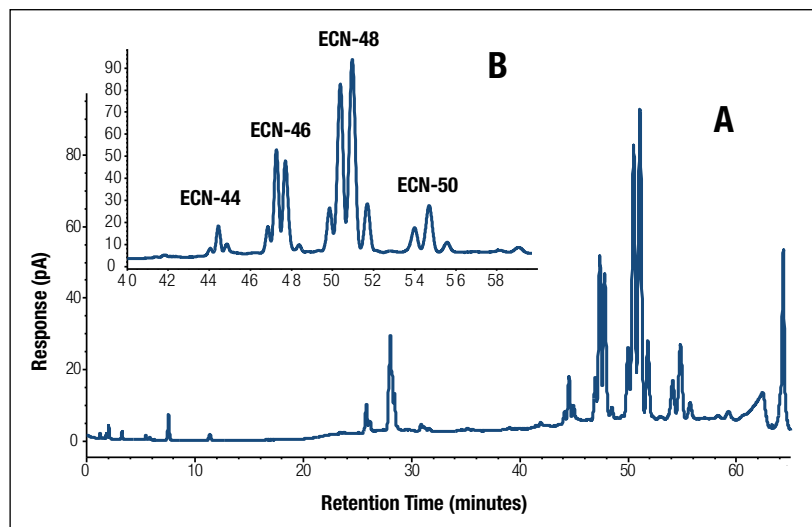


Figure 4A. Chromatogram of a palm oil sample (~ 74 μg on column). Figure 4B. Expanded triglyceride region (40–60 minutes) with the equivalent carbon numbers listed for each set of peaks.

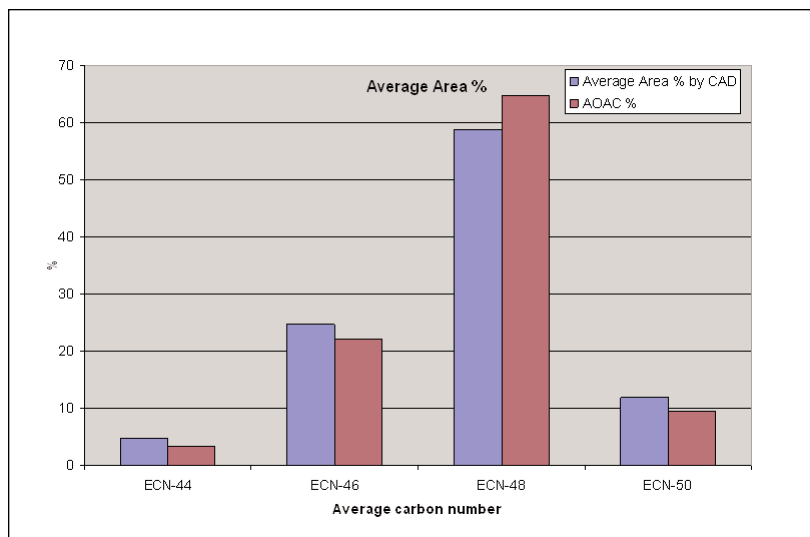


Figure 5. The peak areas for each of the ECNs shown in Figure 4B were added and divided by the total triglyceride peak area for each of the five injections. The average area % is listed for each above. The AOAC results for palm oil as reported by the AOAC Official Method 993.24 are listed. The observed difference is listed to show correlation only. The palm oil sample used in the AOAC method is not the same sample used in this experiment.

Conclusion

The Corona charged aerosol detector, using the HPLC method described above, demonstrates the ability to measure several major lipid components of palm oil. The increased sensitivity and resolution provided by the gradient system and the Corona charged aerosol detector provides improved % relative standard deviation (RSD) over the values observed in AOAC Official Method 933.24. The method also provides a means for quantifying the amount of free fatty acids and mono-, di-, and

triglycerides present in the oil. Even with the limitations noticed due to the matrix effect on palmitic acid, the LOD of the free fatty acid in palm oil is still estimated to be <0.25% w/w when using an injection of ~74 µg of palm oil on column. This method was shown to be more universal than the AOAC HPLC method. It also provides significant advantage over GC methods, which require both extra sample manipulation and derivatization procedures.

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