

# ANALYSIS OF VEGETABLE OILS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY USING EVAPORATIVE LIGHT SCATTERING DETECTION AND NORMAL PHASE ELUENTS

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## INTRODUCTION

Vegetable oils such as safflower, olive, corn, soy, and canola have been employed for a variety of purposes, including as additives for cosmetics and soaps, as health supplements and foodstuff, and for disease prevention. Therefore, the source as well as the nature of the oil is relevant as it is known that harmful oil impurities can have a significant impact on one's health. This work describes the analysis of several edible oils employing state of the art technology, including a Waters® 2424 Evaporative Light Scattering Detector (ELSD) and Alliance® HPLC Technology. HPLC and gas chromatographic methods comprise most of the techniques of analysis done today on edible oils. Other techniques including supercritical fluid chromatography, mass spectrometry, nuclear magnetic resonance spectrometry, and many other techniques have also been employed and have been thoroughly reviewed<sup>1</sup>.

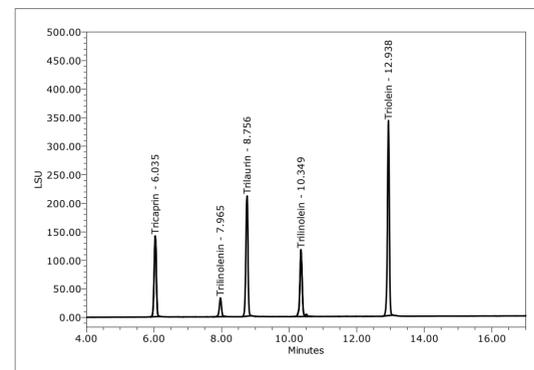


Figure 1. HPLC Separation of 5 Component triglyceride mixture

Analysis of vegetable oils by HPLC presents two distinct challenges. The first challenge is the separation itself, many of the components of vegetable oils are very non polar and comprise a variety of compound classes (fatty acids, triglycerides, waxes, sterols, hydrocarbons, vitamins, and others) resulting in complex mixtures. High resolution separation techniques are important to separate as many of these components from each other as possible. Modern HPLC columns and careful selection of mobile phases have allowed these complex mixtures to be well separated. The second challenge involves detection. Most of the compounds of interest that can be separated by HPLC have no UV chromophore, rendering traditional HPLC-UV detectors unusable. Of the non-chromophore HPLC detectors, refractive index (RI) detectors have been the most widely used for this type of analysis. The RI detector is stable, has a wide linear range, and is simple to operate. Its major drawback is its inability to operate under gradient separation conditions. Gradient separations typically can give better separations resulting in a higher number of resolved peaks. The non-chromophore HPLC detector of choice for gradients is the evaporative light scattering detector (ELSD). This document will show the analysis of several vegetable oils and describe some of the operation principals of ELSD. An example of a 5 component triglyceride separation is shown in figure 1. Conditions for all separations are outlined in the experimental section.

## METHODS

### EXPERIMENTAL

A Waters® Alliance® 2695 system configured with a Waters® 2424 ELSD controlled by Empower™ 2 software was used for all the separations. Details are outlined in table 1.

### SAMPLE PREPARATION

Individual vegetable oils were prepared by weighing ~ 10 mg of the oil into a 20 mL glass container and adding 20 mL of 90:10 acetonitrile/chloroform and mixing well to give a working concentration of 0.50 mg/mL. Diluted samples were transferred to 2 mL vials and kept at room temperature until analysis.

TABLE 1.

HPLC Conditions	
LC System:	Waters® Alliance HPLC System
Detector	Waters® 2424 ELS
Data	Empower™ 2
Column:	2-XBridge™ C18, 3.5 µm 4.6 X 150 mm, connected
Column Temp:	35 °C
Flow Rate:	1.5 mL mL/minute
Injection Volume	25 µL
Mobile Phase A:	Acetonitrile
Mobile Phase B:	Chloroform
Gradient:	10-50% B over 16 minutes Hold 50% B for 2 minutes Return to 10% A over 10 minutes
2424 ELS Detector Conditions	
Nebulizer	Heating at 50% Power
Drift Tube Temp.	65 °C
Nebulizer N <sub>2</sub>	
Pressure	50 psi
Gain	250
Data Rate	10 pps
Time Constant	Normal (0.4 seconds)

## ELSD PRINCIPALS

In evaporative light scattering detection (ELSD), the HPLC solvent stream is nebulized and the droplets formed in the nebulizer are entrained in a stream of gas, which is the same gas flow that was used to nebulize the solvent stream. The droplets are then evaporated to remove the mobile phase. If nonvolatile analyte was present in the solvent stream, "dry" solute particles remain. The particles are carried by the gas to the detection region, where a beam of light intersects the stream of particles and the light scattered by the particles is measured. There are three separate regions of an ELS detector (nebulization, desolvation, detection). In all ELSD's, these three regions are positioned so that the HPLC effluent is nebulized, mobile phase is evaporated and removed, and the analytes alone are sent to the detector. Users control the rate of evaporation by manipulation of the nebulizer and drift tube temperatures along with the nebulizing gas pressure. The actual settings are dependant on the composition and flow rate of the mobile phase. Non-optimal instrument settings can adversely affect results. Figure 8 shows an example of an identical sample at two different drift tube temperatures. A significant reduction (~50%) in signal can be seen in the sample with the drift tube temperature set too high.

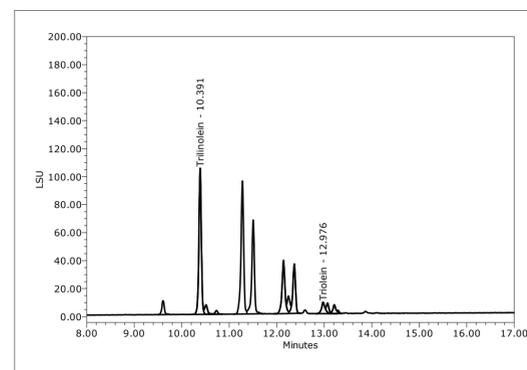


Figure 2. HPLC Separation of Soybean Oil

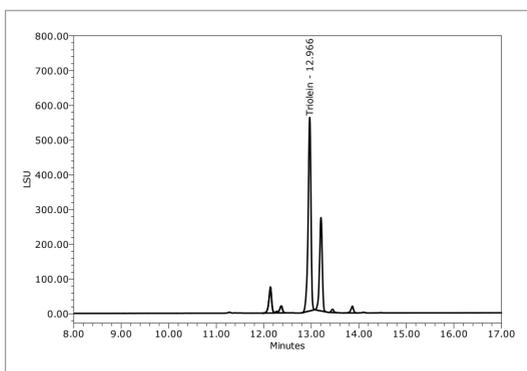


Figure 3. HPLC Separation of Olive Oil

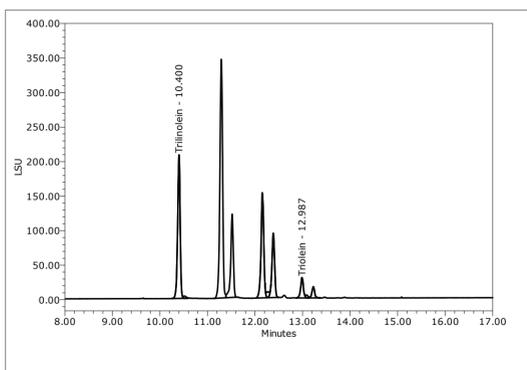


Figure 4. HPLC Separation of Corn Oil

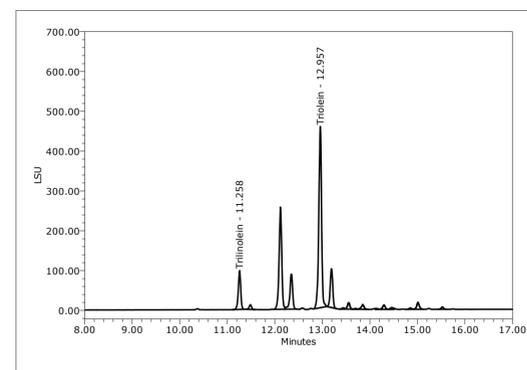


Figure 5. HPLC Separation of Peanut Oil

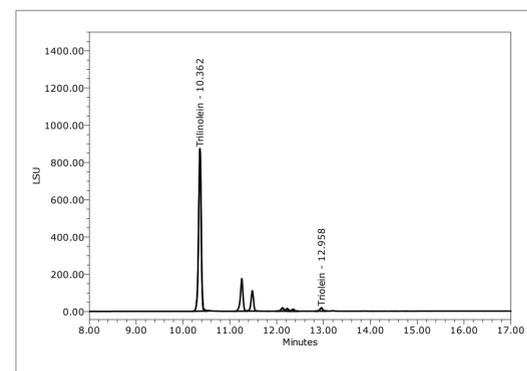


Figure 6. HPLC Separation of Safflower Oil

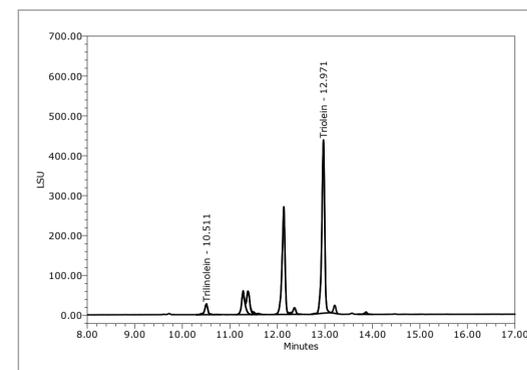


Figure 7. HPLC Separation of Canola Oil

## RESULTS AND CONCLUSIONS

Differences in the chromatographic patterns of each of the oils analyzed was noted (figures 2-7). The combination of the dual 3.5 µm particle size XBridge columns coupled with the CH<sub>3</sub>CN/Chloroform gradient provided good resolution of the oil components. When compared to each other, all of the oils had some peaks in common (Table 2) although each of the oils had a unique chromatographic fingerprint. Differentiation at this level is important when using this kind of chromatographic approach as it allows users of these products (either as raw materials or finished products) to quickly tell one oil from another (figure 9 for example). Differences from year to year, growing region to growing region, and oil adulteration could also be determined from this kind of analysis. The results show that HPLC analysis coupled with ELS detection can provide sufficient information to understand the nature and purity of a vegetable oil in a single run.

TABLE 2.

Sample Name	Area Counts	
	Trilinolein LSU	Triolein LSU
0.50 mg/mL Soy Oil	371153	41925
0.50 mg/mL Corn Oil	767522	121203
0.50 mg/mL Olive Oil	Not Found	2326458
0.50 mg/mL Peanut Oil	351874	1846721
0.50 mg/mL Safflower Oil	3341047	73522
0.50 mg/mL Canola Oil	119002	1852941
Sample Name	Area %	
	Trilinolein	Triolein
0.50 mg/mL Soy Oil	24.8	2.8
0.50 mg/mL Corn Oil	20.1	3.2
0.50 mg/mL Olive Oil	Not Found	60.2
0.50 mg/mL Peanut Oil	8.4	43.9
0.50 mg/mL Safflower Oil	71.3	1.6
0.50 mg/mL Canola Oil	3.1	47.7



Waters 2424 ELSD

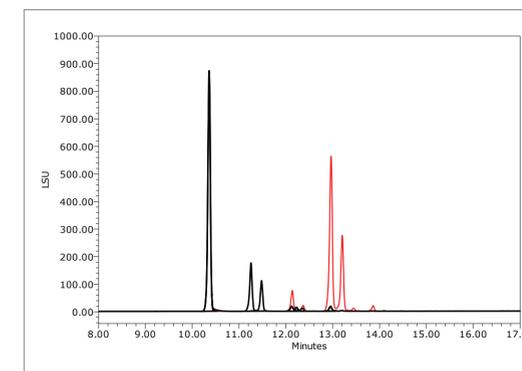


Figure 9. Overlaid HPLC Separation of Safflower Oil (black) and Olive Oil (red).



Waters 2424 ELSD

### References

- R. Aparicio, R. Aparicio-Ruiz J. Chromatography A 881 (2000) 93-104