

응용 자료

## Analysis of Vegetable Oils by High Performance Liquid Chromatography Using Evaporative Light Scattering Detection and Normal Phase Eluents

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## Abstract

This application note describes the analysis of several edible oils that employ state-of-the-art technology, including Waters 2424 Evaporative Light Scattering Detector (ELSD) and Alliance HPLC Technology.

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## Introduction

Vegetable oils – such as safflower, olive, corn, soy, and canola – have been employed for a variety of purposes, including: additives for cosmetics, soaps, health supplements, and foodstuffs; as well as disease prevention. The source and nature of the oil is relevant, as harmful oil impurities can have a significant impact on health and wellbeing. This application note describes the analysis of several edible oils that employ state-of-the-art technology, including Waters 2424 Evaporative Light Scattering Detector (ELSD) and Alliance HPLC System Technology.

High Performance Liquid Chromatography (HPLC) and gas chromatographic methods comprise most analysis techniques 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 thoroughly reviewed.<sup>1</sup>

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) that result 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; this renders 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 give better separations, which result in a higher number of resolved peaks. The non-chromophore HPLC detector of choice for gradients is the Evaporative Light Scattering Detector (ELSD). This application note will show the analysis of several vegetable oils and describe some of the

operation principals of ELSD.

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## Experimental

A Waters Alliance e2695 HPLC System configured with a Waters 2424 ELSD, and controlled by Waters Empower 2 Software was employed for data collection and analysis. Commercially prepared soy, corn, olive, peanut, safflower, and canola oils were purchased at a local grocery store. Triglyceride standard materials were obtained from Sigma-Aldrich. Acetonitrile and chloroform were of HPLC grade and obtained from Fisher Scientific.

### HPLC conditions:

LC system:	Alliance HPLC
Detector:	2424 ELSD
Data:	Empower 2 Software
Column:	Two XBridge C <sub>18</sub> 3.5 μm 4.6 x 150 mm, connected in series
Column temperature:	35 °C
Flow rate:	1.5 mL/min
Injection volume:	25 μL
Mobile phase A:	Acetonitrile
Mobile phase B:	Chloroform
Gradient:	10% to 50% B over 16 min Hold 50% B for 2 min Return to 10% A over 10 min

## 2424 ELS Detector conditions:

Nebulizer:	Heat at 50% power
Drift tube temperature:	65 °C
Nebulizer gas:	N <sub>2</sub>
Pressure:	50 psi
Gain:	250
Data rate:	10 pps
Time constant:	Normal (0.4 sec)

## Sample preparation

Individual oil samples were prepared by weighing approximately 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.

## ELSD parameters

In evaporative light scattering detection, 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 non-volatile 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, and 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 dependent on the composition and flow rate of the mobile phase. Non-optimal instrument settings can adversely affect results. Figure 1 shows an example of an identical sample at two different drift tube temperatures. A 50% reduction in signal can be seen in the sample with the drift tube temperature set too high.

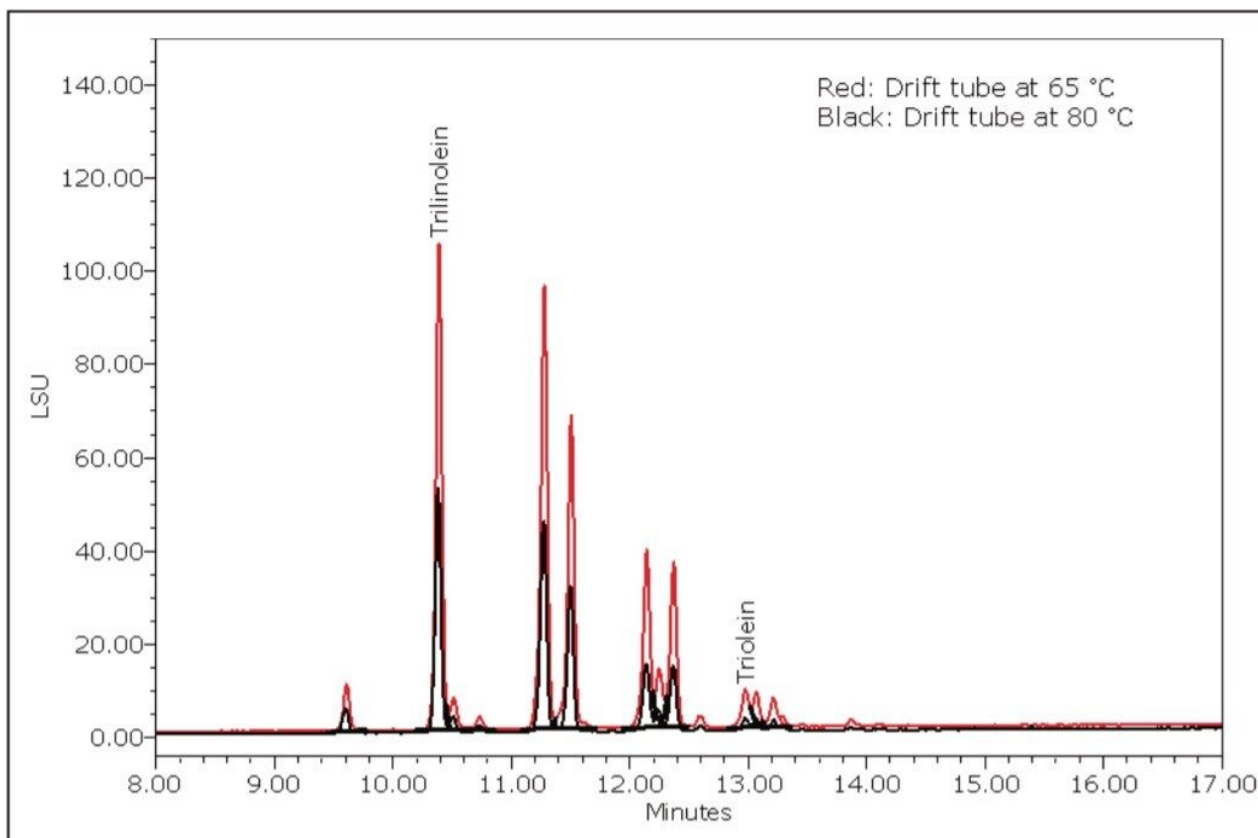


Figure 1. HPLC separation of soybean oil at two different drift tube temperatures.

## Results and Discussion

The two XBridge C<sub>18</sub> 3.5  $\mu$ m 4.6 X 150 mm Columns were connected in series and combined with the acetonitrile/chloroform gradient. This provided good resolution of the triglycerides mixture, as shown in Figure 2. Each of the oils analyzed has a unique chromatographic fingerprint, as shown in Figures 3 to 7. However, all of the oils had some peaks in common when compared to each other, as shown in Table 1. Differentiation at this level is important as it allows users of these products (either as raw materials or finished products) to quickly tell one oil from another, as shown in Figure 8. Differences from year to year, growing region to growing region, and oil adulteration could also be determined from this kind of analysis.<sup>2</sup>

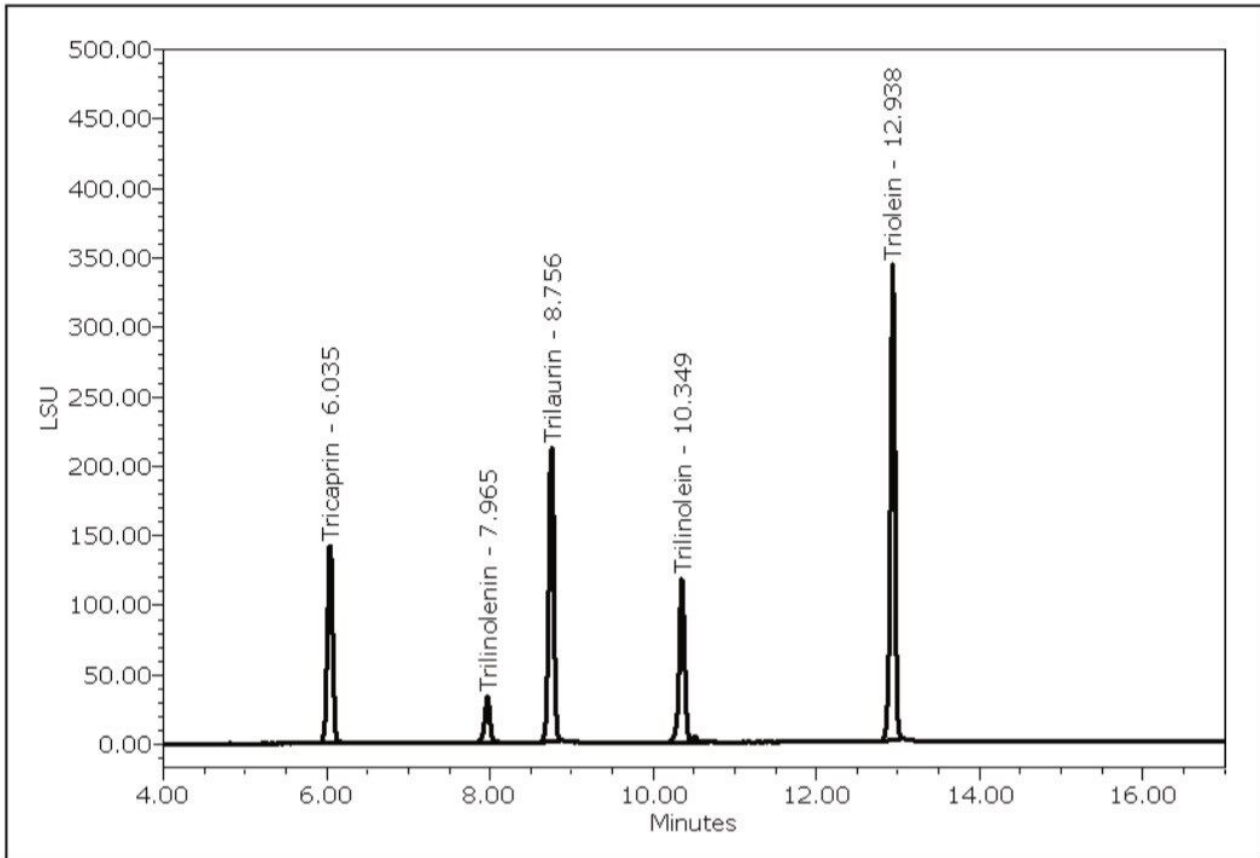


Figure 2. HPLC separation of five component triglyceride mixtures.

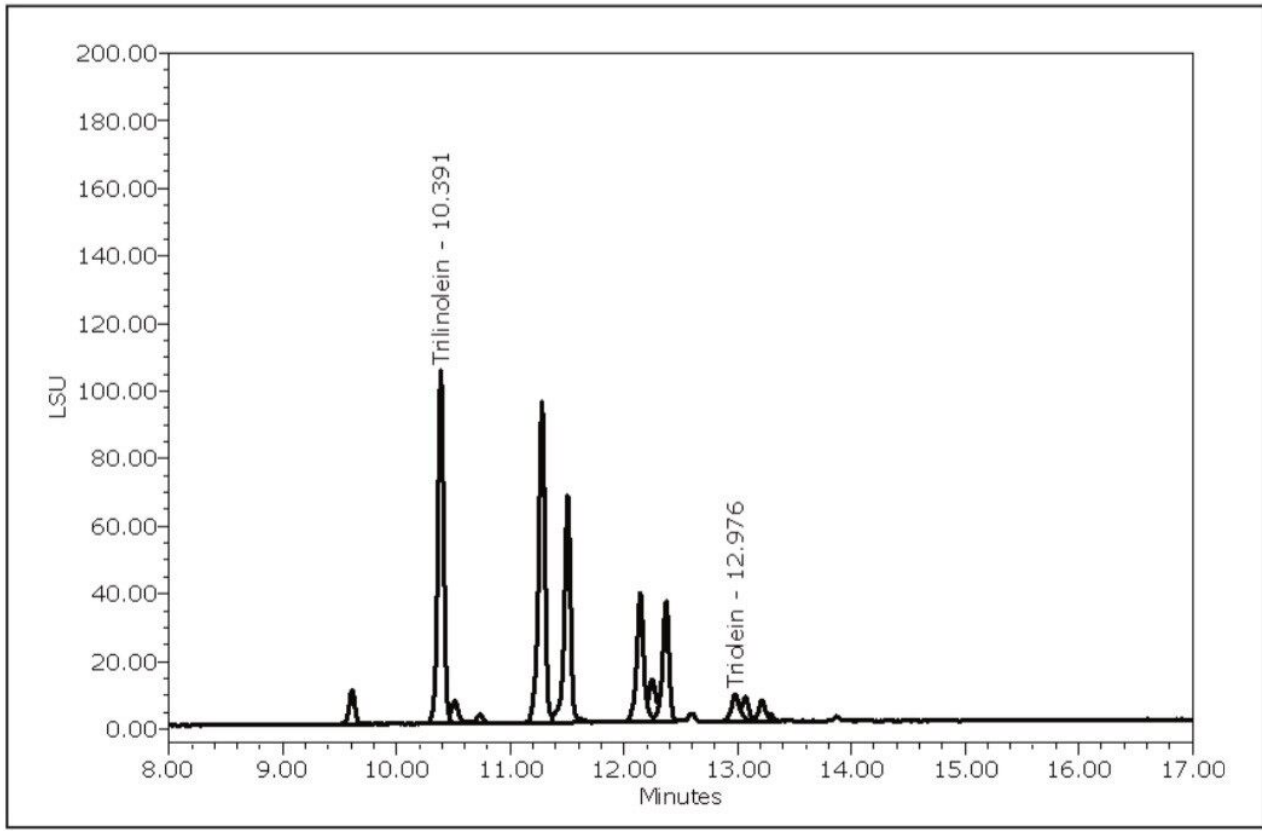


Figure 3. HPLC separation of soybean oil.

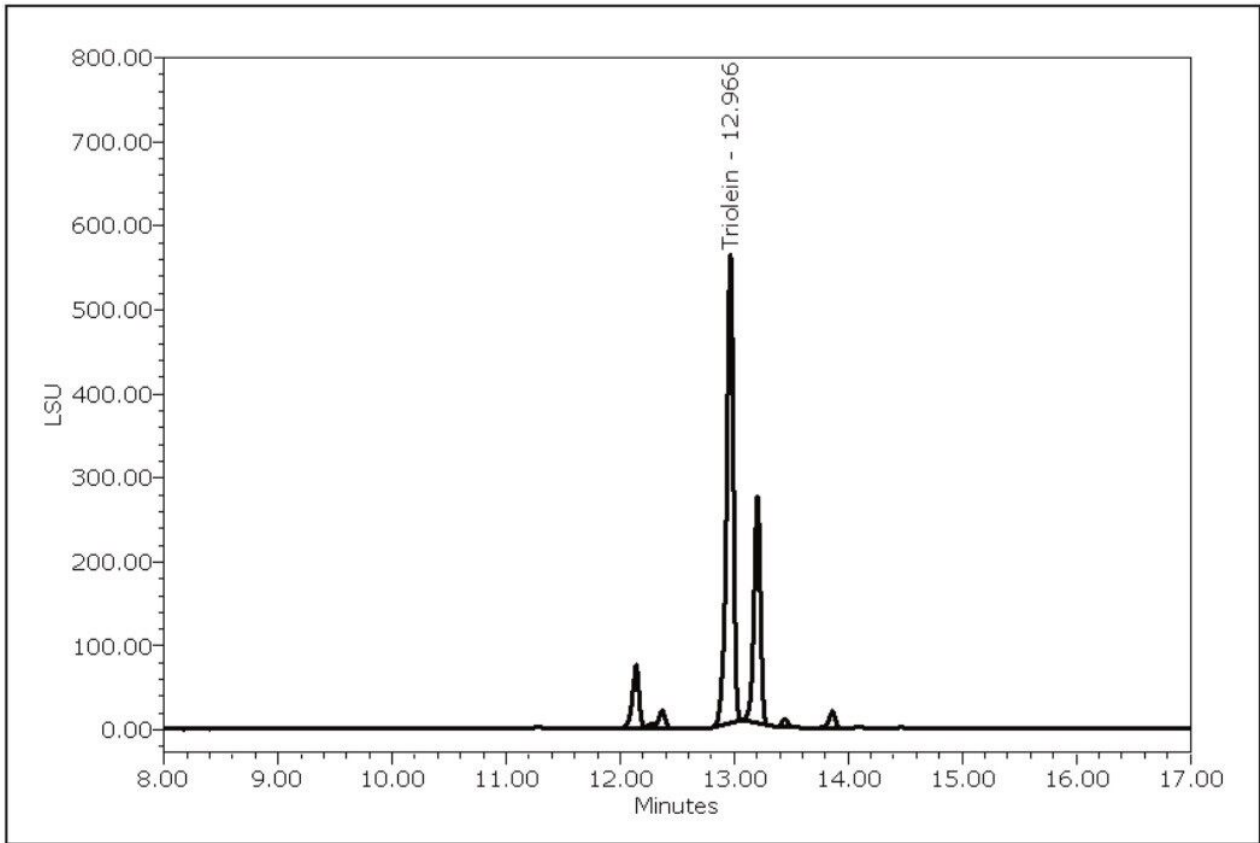


Figure 4. HPLC separation of olive oil.



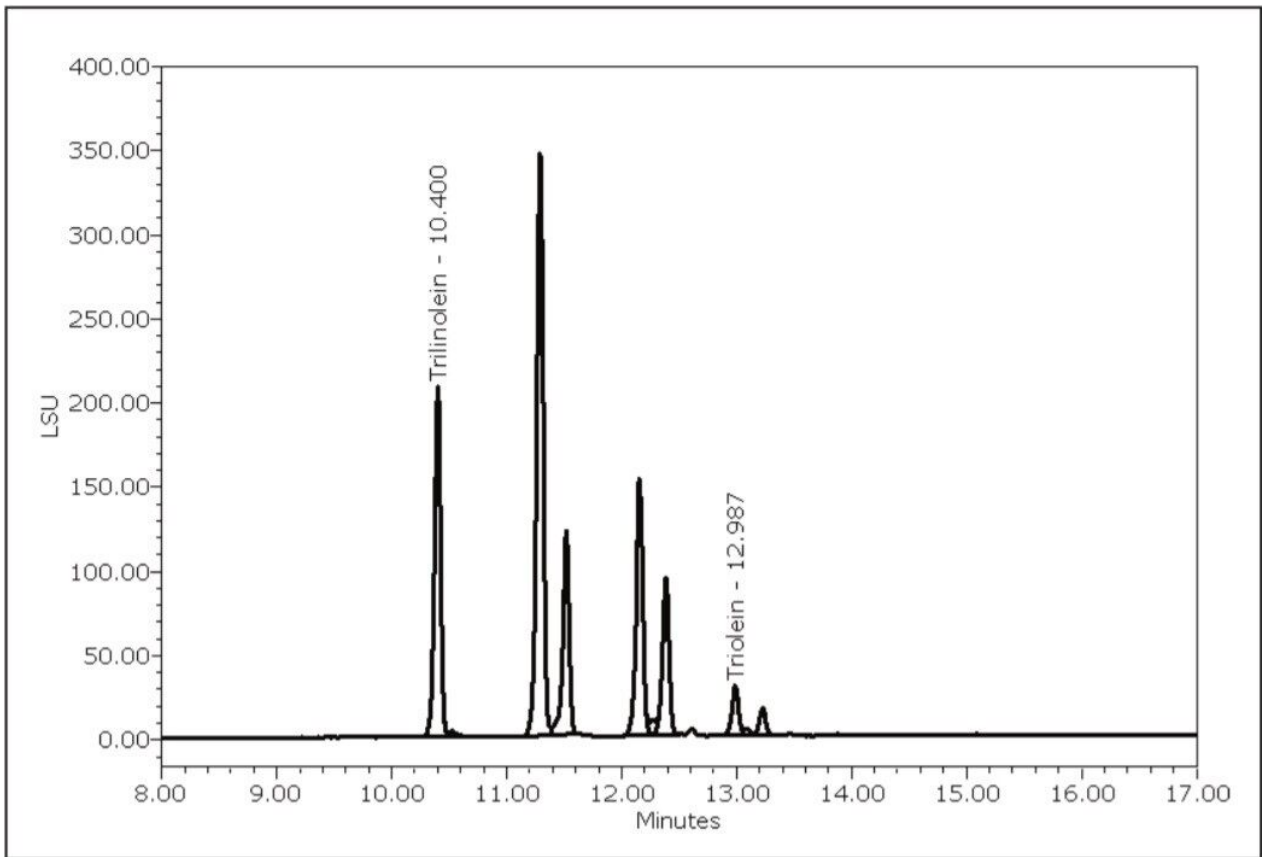


Figure 5. HPLC separation of corn oil.

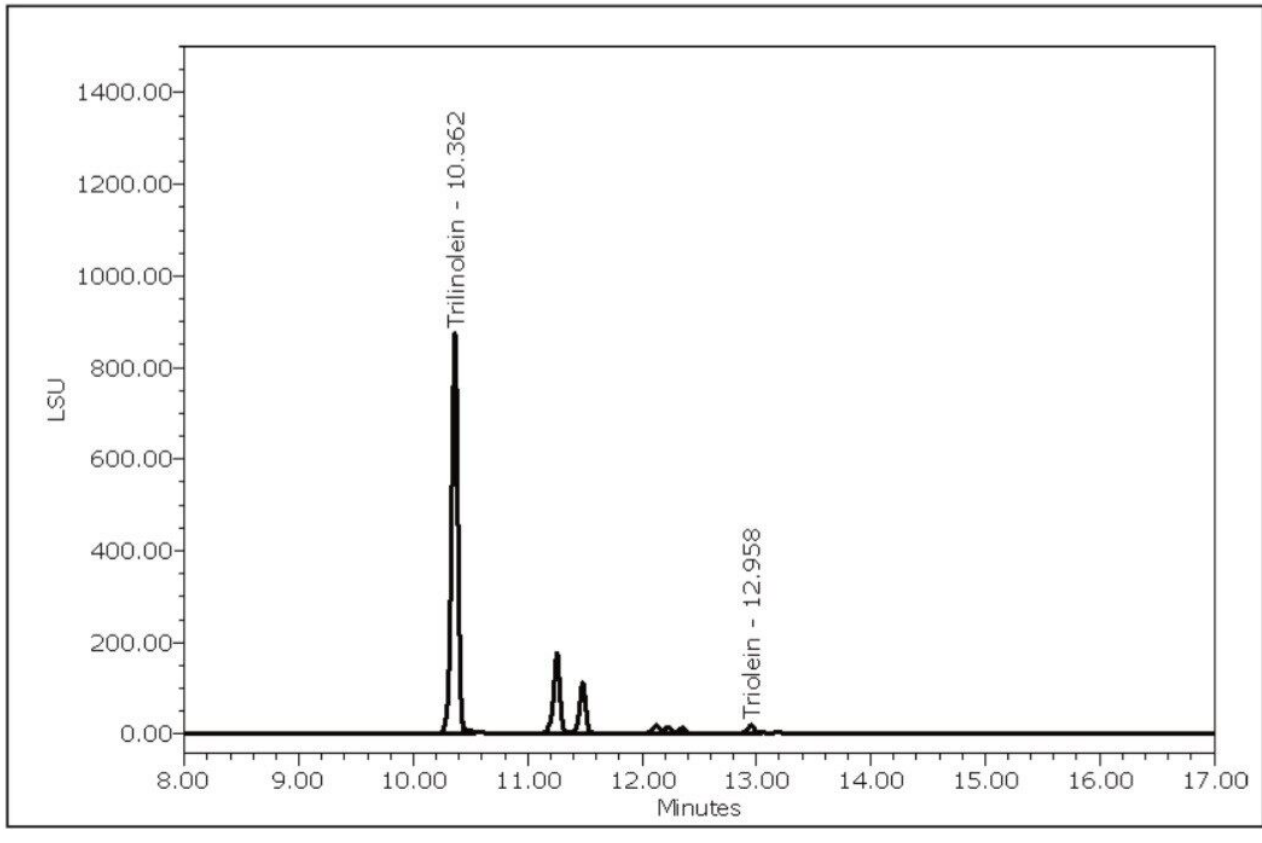


Figure 6. HPLC separation of safflower oil.

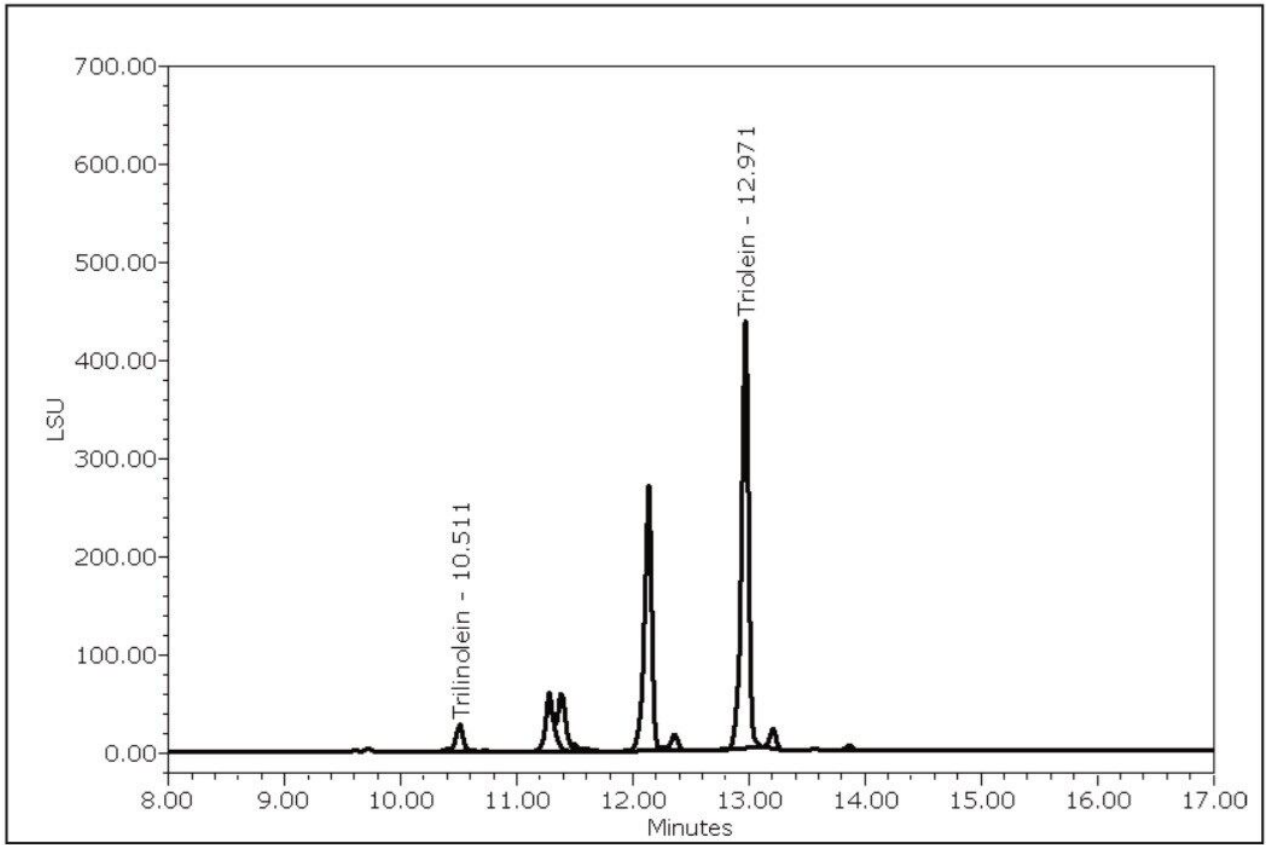


Figure 7. HPLC separation of canola oil.

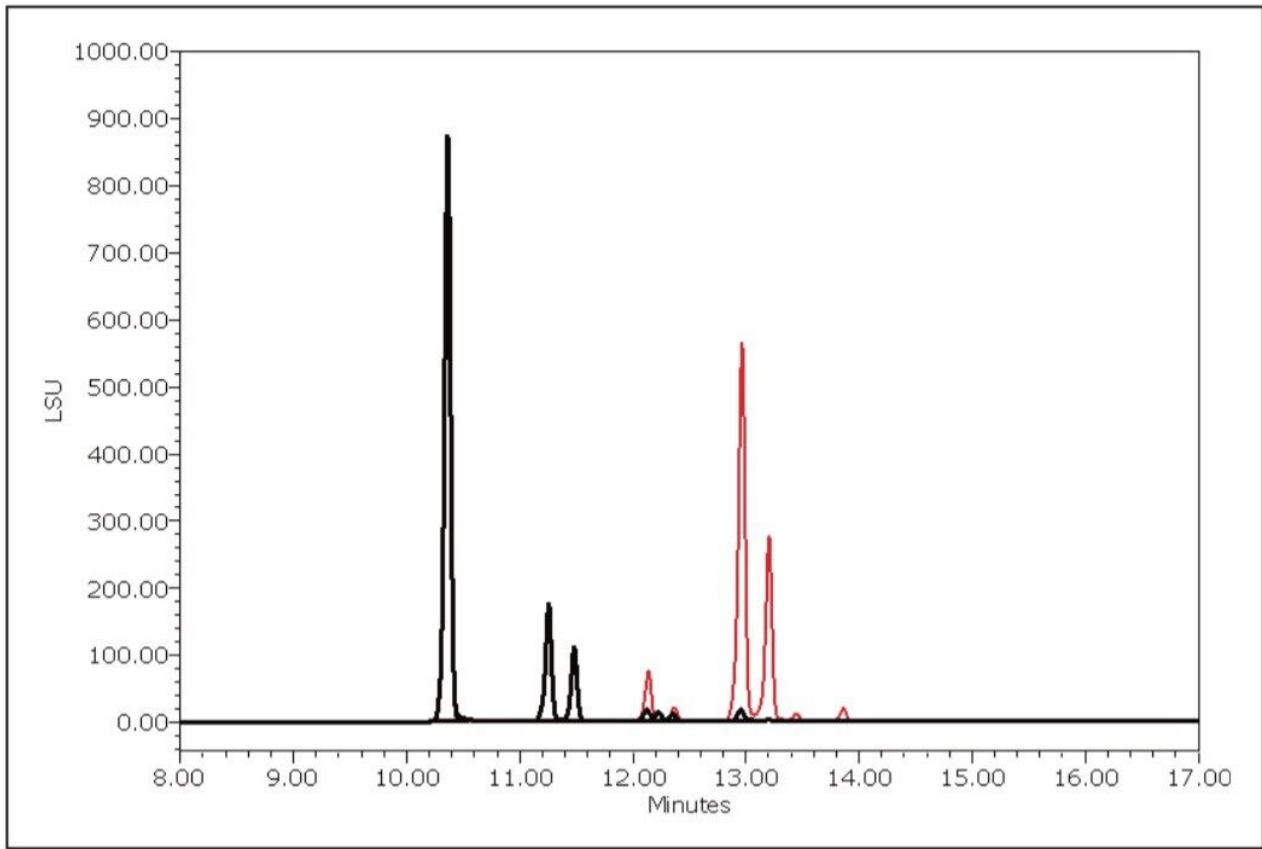


Figure 8. Overlaid HPLC separation of safflower oil (black) and olive oil (red).

	Area Counts	
	Trilinolein LSU	Triolein LSU
<b>Sample Name</b>		
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

	Area %	
	Trilinolein	Triolein
<b>Sample Name</b>		
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

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## Conclusion

- The non-chromophore HPLC detector of choice for gradients is the evaporative light scattering detector (ELSD).
- ELSD nebulizer and drift tube temperatures and nebulizing gas pressure are critical parameters. The actual settings are dependent on the composition and flow rate of the mobile phase. Optimization of these settings is an important part of method development. Non-optimal instrument settings can adversely affect results.
- The results show that gradient HPLC analysis coupled with ELS detection provide sufficient information to understand the nature and purity of a vegetable oil in a single run.
- The Alliance e2695 HPLC System configured with a 2424 ELSD controlled by Empower 2 Software provide a reliable, easy-to-use system that is ideally suited to the analysis of complex mixtures of compounds lacking a UV chromophore.

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## References

1. R. Aparicio, R. Aparicio-Ruiz *J. Chromatography A* 881(2000) 93-104
2. Trinite, D. A. Mazza, D. Not All Vegetable Oils are Created Equal: An Evaluation Using Evaporative Light Scattering Detection and Normal Phase Eluents. Waters Application Note: 720001121EN

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## Featured Products

Alliance HPLC System <<https://www.waters.com/534293>>

2424 Evaporative Light Scattering (ELS) Detector <<https://www.waters.com/514428>>

Empower 3 Chromatography Data Software <<https://www.waters.com/10190669>>

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