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應用手冊

Not All Vegetable Oils are Created Equal: An Evaluation Using Evaporative Light Scattering Detection and Normal Phase Eluents

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#### Abstract

This application note illustrates analysis of several edible oils employing state of the art technology, including a Waters 2420 Evaporative Light Scattering Detector (ELSD).

#### 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 are relevant as it is known that harmful oil impurities can have a significant impact on one's health.

A vegetable oil will have a unique triglyceride composition that analyzed by HPLC can provide a distinctive peak pattern. Fingerprint pattern permits the analyst to determine the source of the oil and the degree of adulteration, if any. The detection mode is critical in obtaining as much information as possible per run. While triglycerides can be analyzed by HPLC, they present poor UV/VIS response because they lack strong chromophores, so alternatives to LC/UV are sought. In the past scientists have used flame ionization detection based chromatography for the evaluation of vegetable oils, but evaporative light scattering detection has been embraced by laboratories worldwide because of present day instrument performance. To this end, this document illustrates analysis of several edible oils employing state of the art technology, including a Waters 2420 Evaporative Light Scattering Detector (ELSD).

# Experimental

A Waters Alliance 2695 System with a Waters 2996 Photodiode Array Detector and a 2420 ELSD were employed in this work. Two Symmetry  $C_{18}$  columns, 5  $\mu$ m, 3.9 x 150 mm at 30 °C were used. The gradient conditions were as follows: initial conditions 80/20 Eluent A/Eluent B (where Eluent A = acetonitrile and Eluent B = methylene chloride) to 40/60 in 72 minutes. The injection volume was 50  $\mu$ L and the flow rate was 0.57 mL/min. The ELSD conditions were set up as outlined here:

Nebulizer:	Low Flow Nebulizerat 50%
Drift tube temp.:	65 °C
Nebulizer pressure	26.6 psi
Gain:	50
Time constant:	1

Samples of olive oil, soy oil, safflower oil, corn oil and canola oil were analyzed in this evaluation. They were each dissolved in both 100% methylene chloride and 80/20 acetonitrile/methylene chloride to evaluate peak patterns and sample solvent effects.

# **Principles of Operation**

An Evaporative Light Scattering Detector has three sections as depicted in Figure 1: a nebulization section where the column eluent comes in contact with the vaporization gas forming an aerosol, a desolvation section where the aerosol droplets are heated and the solvent evaporates leaving dry particles, and a detection section where particle scatter is measured. The formation of aerosol droplets occurs in the nebulizer, the desolvation takes place in the drift tube, and the scatter is measured in the scatter chamber. All areas are heated, and while the nebulizer and drift tube can be controlled by the user, the scatter chamber is kept at constant temperature to avoid product performance issues due to sample deposition within it.

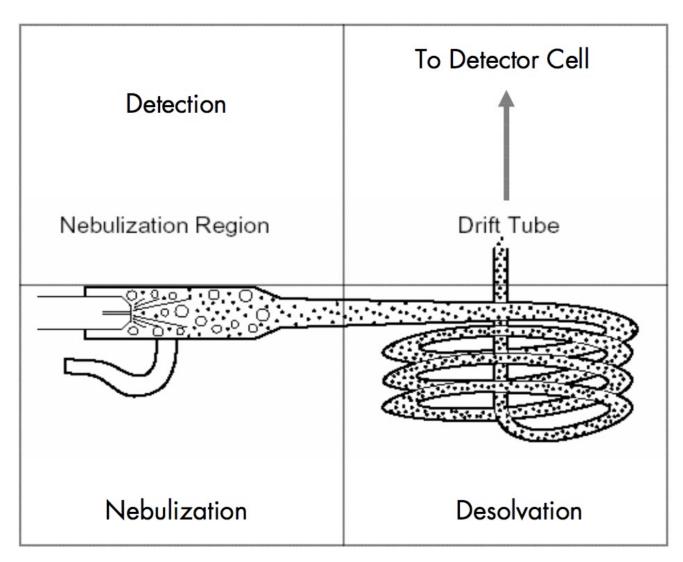


Figure 1. Evaporative light scattering detector operation.

#### Results and Discussion

The objective of this study was to evaluate differences in chromatographic patterns for several vegetable oils using state-of-the-art technology. The evaluation included oils from the same plant (available through several sources), as well as oils from different plants.

While scientists often seek a strong solvent to dilute their sample in due to throughput constraints, this can have an impact on the resolution. To this end, effects of sample solvent on the final chromatographic results are presented in Figures 2 and 3. Briefly, Figure 2 shows the results for a soy oil sample, where the oil is diluted in methylene chloride. As can be seen, the chromatographic resolution is poor -especially for the early eluting triglycerides. In particular, some of the components are not even retained in the column. These effects are critical

as these chromatographic results are used for determining batch differences in oil deliveries, as well as for pinpointing product adulteration. Conversely, Figure 3 depicts results for the same soy oil sample, this time diluted in initial mobile phase conditions (80/20 acetonitrile/methylene chloride). This new sample dilution scheme significantly improves chromatographic resolution, confirming that the nature and composition of the sample solvent is crucial to chromatographic performance.

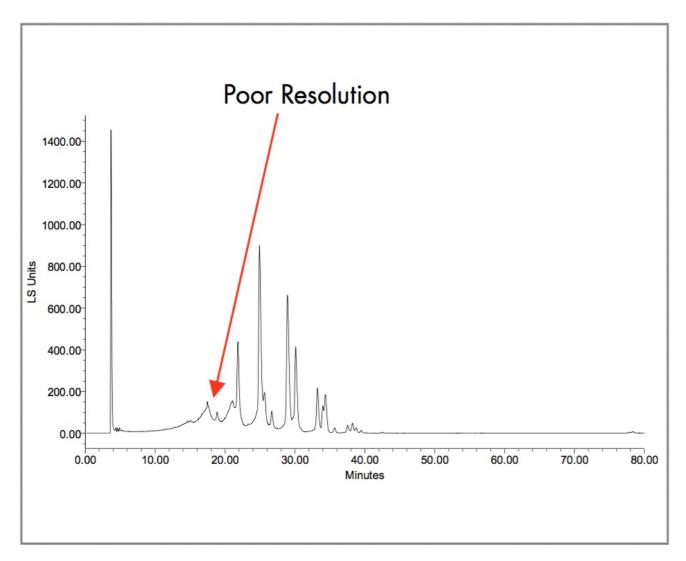


Figure 2. Soy oil diluted in 100% methylene chloride. Strong sample solvent effects are seen in the early part of the chromatogram.

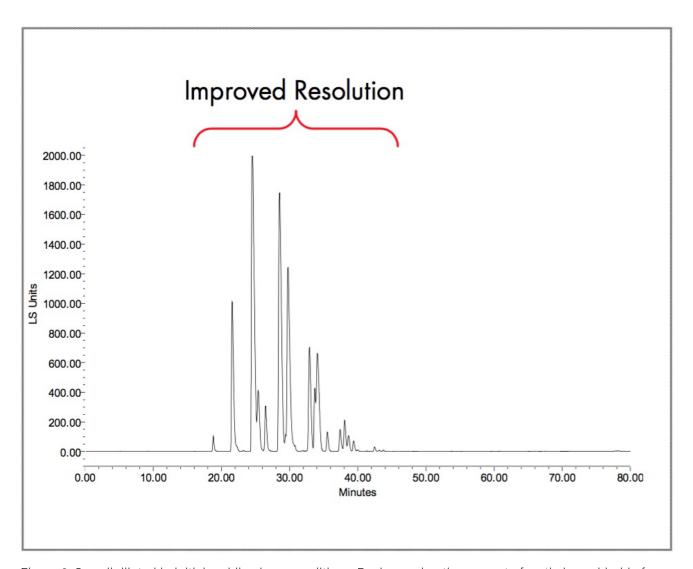
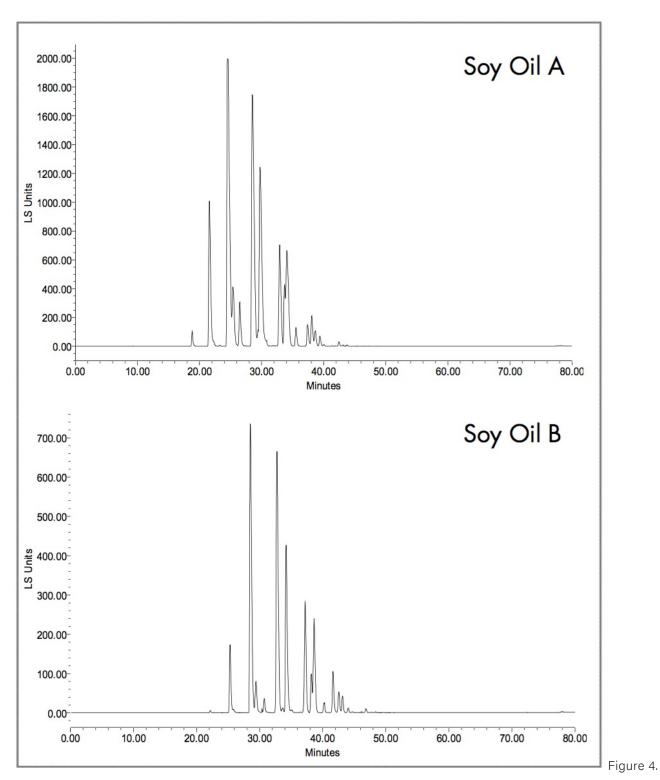


Figure 3. Soy oil diluted in initial mobile phase conditions. By decreasing the percent of methylene chloride for sample dilution, improved chromatography occurs.

Figure 4 shows the chromatographic results for soy oils from different sources. It is clear that the peak pattern is not the same in both soy oil samples, indicating that the differing harvest season and year coupled with varying environmental conditions tend to affect the final product supplied to manufacturers, distributors and consumers.



Chromatographic results with two samples of soy oil, illustrating the differences in chromatographic patterns due to raw material composition of several vegetable oils run under the same conditions. As seen, there is a significant difference in peak patterns when evaluating safflower, olive, corn, soy, and canola oils. These results are noteworthy because when carrying out quality control analysis of raw materials or finished products, the

chromatographic approach taken here is relevant to those that differentiate amongst oils coming from distinct
regions of the world or are tasked with isolating an adulterated sample. Further, the results show that it is
possible to obtain sufficient information to characterize the nature and purity of a vegetable oil in a single run,
thereby increasing productivity in the laboratory.

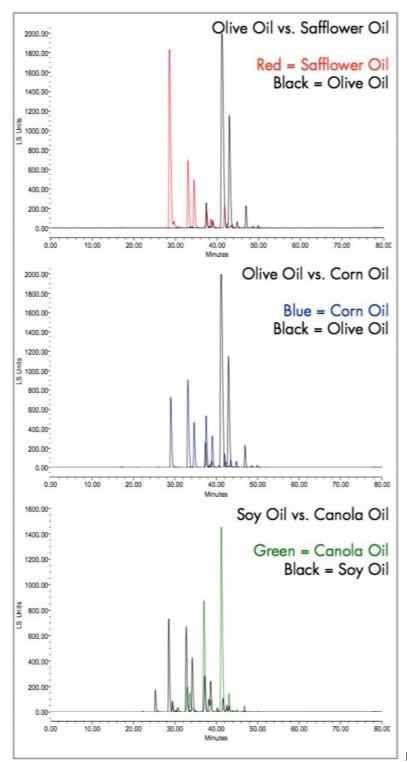


Figure 5. Chromatographic results indicating

the peak pattern differences amongst oils of different triglyceride composition.

# Conclusion

The work presented illustrates a useful LC/ELSD approach to analyze vegetable oils using organic solvents in an effective manner. We have demonstrated that a Waters 2420 ELSD is a necessary complementary detector in today's laboratories for finding more information per single run, especially when analyzing UV/VIS transparent compounds. The results indicate that sample dilution is best carried out when employing initial mobile phase compositions. Further, this approach permits the identification of vegetable oil origin and potential adulteration components as peak patterns are well defined.

## References

 Neff W.E. and Byrdwell W.C. Soybean. Oil Triacylglycerol Analysis by Reversed-Phase High-Performance Liquid Chromatography Coupled with Atmospheric Pressure Chemicallonization Mass Spectrometry. *JAOCS*, Vol. 72, No. 10, 1995, p.1185–1191.

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