Application Note SI-01231

High Resolution Analysis of Triglycerides in Vegetable Oils by HPLC with ELSD

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Introduction

The Varian evaporative light scattering (ELS) detector is universal and its response is not dependent on the optical properties of the compound. Consequently, its good discriminating power and sensitivity is well suited to compounds such as triglycerides that possess weak or no UV chromophores.

Triglycerides (TAGs) are triesters of glycerol and three fatty acids, with typical chain lengths of 18 carbon atoms and degrees of unsaturation varying between 0 and 6. Typical fatty acids are stearic, oleic (cis-9) and linoleic (cis, cis-9, 12). Vegetable oils, which are primarily composed of triglycerides, possess a unique triglyceride fingerprint that can be used to determine their origin and quality. The low volatility of triglycerides prevents them being analyzed directly by gas chromatography. An indirect method involving transesterification of the triglycerides to methyl esters via a derivatization step has been used, but this can be problematic for complex mixtures. The IUPAC method for triglyceride analysis uses HPLC coupled with refractive index detection. Since this method uses isocratic elution, run times are generally long, especially for mixtures containing long chain triglycerides. HPLC methods employing gradient elution with UV detection have provided faster separations than refractive index. However, due to the low wavelengths necessary for triglyceride detection (e.g. 210 nm), UV analysis is very susceptible to baseline drift under gradient elution. In addition, the choice of mobile phase solvents is limited at lower wavelengths.

All of these difficulties are overcome by the Varian ELS detector because it is independent of the optical properties of a compound, detects any compound that is less volatile than the mobile phase and is compatible with a wide range of solvents displaying excellent baseline stability with gradient elution.

Instrumentation

Column: C18 5 µm, 250 x 4.6 mm
Detection: Varian ELSD (neb=25 °C, evap=50 °C, gas=1.4 SLM)

Materials and Reagents

Eluent A: ACN
Eluent B: DCM

Symbol	Triglyceride	No of Carbons
La	Lauric acid	12
P	Palmitic acid	16
Po	Palmitoleic acid	16
S	Stearic acid	18
O	Oleic acid (cis-9)	18
L	Linoleic acid (cis, cis-9, 12)	18
Ln	α-Linolenic acid	18

Sample Preparation

Sample: 1 mg olive and sunflower oil/mL

Conditions

Flow Rate: 1.0 mL/min
Injection Volume: 20 µL
Gradient: 30-50 % B in 40 min, 50-90 % in 2 min, hold 3 min

Results and Discussion

Figures 1 and 2 show the very different profiles of olive and sunflower oil, with olive oil having a higher percentage of triolein (OOO) than sunflower oil. This type of information can be used to identify adulterated oils. Likewise, the compositional differences between sesame and walnut oil can be determined by ELSD. Sesame and walnut oil are both good sources of linolenic acid (L), but their compositions are very different, as shown in Figures 3 and 4. Sesame oil displays a complex triglyceride fingerprint compared to the simpler profile of walnut oil, with sesame oil displaying a higher proportion of oleic acid.

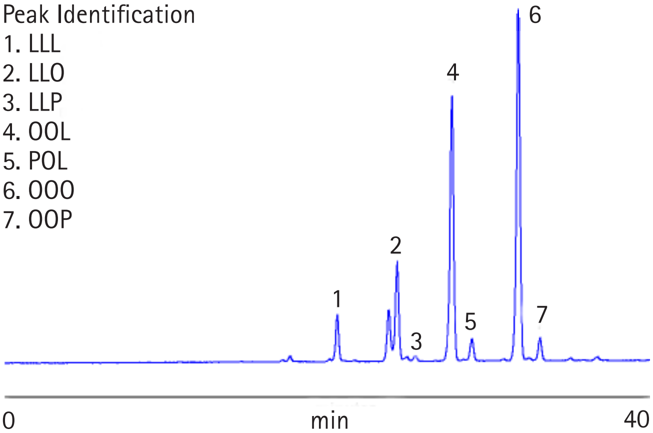

Figure 1. Low percentage of triolein in sunflower oil revealed by ELS detection.

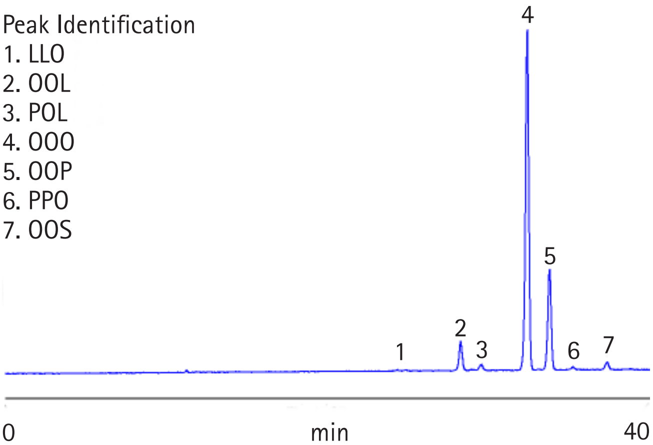

Figure 2. ELSD makes plain the high percentage of triolein in olive oil.

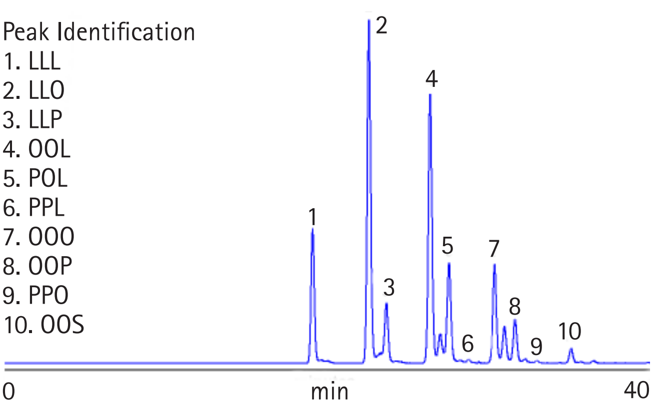

Figure 3. The complex triglyceride composition of sesame oil uncovered by ELS detection.

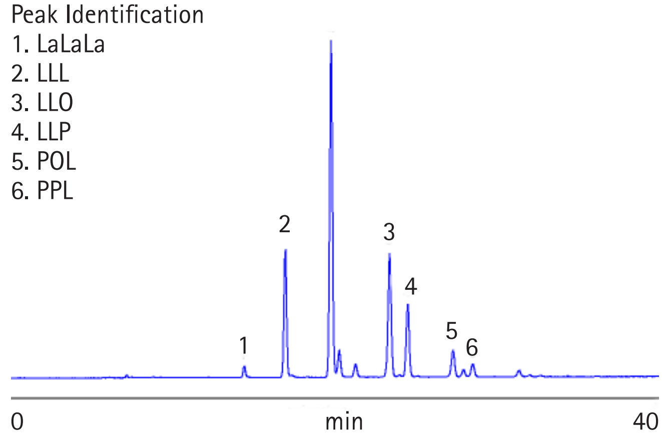

Figure 4. Simpler trigyceride fingerprint of walnut oil with low concentration of oleic acid displayed by ELSD.

Conclusion

In order to obtain these high resolution separations of oils with complex triglyceride profiles, gradient elution is required using organic solvents such as DCM; an approach that is not feasible with RI or UV detection. The use of the Varian ELS detector to analyze triglycerides allows analysts to perform faster chromatography without compromising resolution, making ELS a better alternative to UV and RI detection.

The Varian ELS detector surpasses other ELSDs for low temperature HPLC applications with semi-volatile compounds. Its innovative design represents the next generation of ELSD technology, providing optimum performance across a diverse range of HPLC applications. The Varian ELS detector's unique gas control permits evaporation of high boiling solvents at very low temperatures. For example, 100 % water at a flow rate of 5 mL/min can be removed at 30 °C. The novel design of the Varian ELS detector provides superior performance compared to competitors' detectors for the analysis of semi-volatile compounds.

These data represent typical results. For further information, contact your local Varian Sales Office.

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