



# Analysis of triglycerides in vegetable oils using the Agilent 1260 Infinity Analytical SFC System with evaporative light scattering detection

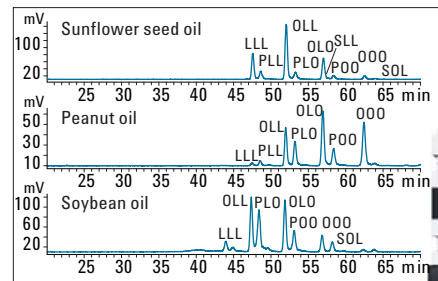
## Application Note

Food

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### Abstract

Supercritical fluid chromatography (SFC) in combination with evaporative light scattering detection (ELSD) is a valuable technique for the determination of triglyceride composition of vegetable oils. In comparison to GC, triglycerides are separated at much lower temperature and compared to HPLC, different selectivities and shorter analysis times are obtained with SFC. Using octadecyl silicagel (ODS), a reversed phase-type separation is obtained which gives separation of triglycerides based on carbon number and number of double bonds. Using a silver loaded column, triglycerides are separated based on the degree of unsaturation. Both separation mechanisms provide complementary information, as illustrated by the analysis of sunflower seed oil, peanut oil, and soybean oil.



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## Introduction

The potential of supercritical fluid chromatography for the separation of triglycerides has been demonstrated for many years<sup>1-3</sup>. Using a reversed phase stationary phase, such as ODS, the separation is similar to that obtained in reversed phase HPLC. Separation is based on carbon number (total number of carbons in fatty acids) and on the total number of double bonds. Using a silver loaded column, separation is primarily based on the degree of unsaturation (total number of double bonds). These two separation mechanisms are complementary.

This Application Note demonstrates the SFC separation of triglycerides in three vegetable oils. Detection was done using evaporative light scattering detection (ELSD) in series with UV/DAD detection.

## Experimental

### Solutions

Sunflower seed oil, peanut oil, soybean oil reference oils, tripalmitin (PPP), triolein (OOO), and trilinolein (LLL) standards were purchased from Sigma-Aldrich (Bornem, Belgium). The oils were dissolved in chloroform at 5% (50 mg/mL) level.

## Instrumentation

Analyses were performed on an Agilent 1260 Infinity Analytical SFC System combined with an Agilent 1260 Infinity ELSD Evaporative Light Scattering Detector. The ELSD was coupled to the SFC module using a procedure similar to the one used for SFC-MS<sup>4</sup>. The system configuration is summarized in Table 1.

The addition of a make-up flow after the backpressure regulator, together with additional heating at the entrance of the ELSD, was found necessary to obtain good sensitivity, reproducibility, and avoid solute deposition in the transfer capillary. Experiments show that switching off make-flow or heating immediately results in low sensitivity and unstable baseline in ELSD detection.

Part number	Description
G4309A	Agilent 1260 Series Analytical SFC System
G1310B	Agilent 1260 Infinity Isocratic Pump (Make-up Flow)
G4260B	Agilent 1260 Infinity Evaporative Light Scattering Detector
AG1	Caloratherm Available through RIC <sup>1,2</sup>
AG004	Pre-Heater Available through RIC <sup>1,2</sup>

<sup>1</sup>Contact info@richrom.com for more information.

<sup>2</sup>Alternatively, the heat exchanger of a G1316A thermostatted column compartment can be used to heat the transfer line to the ELSD.

**Table 1**  
System modules.

## Experimental conditions

Analyses were performed on two different stationary phases: a ZORBAX SB-C18 and a Chromspher 5 Lipids silver loaded column. For the reversed phase separation, three ZORBAX SB-C18 columns were coupled in series. The experimental conditions are summarized in Tables 2 and 3.

Conditions	
Column:	Agilent ZORBAX SB-C18: 3x (4.6 × 250 mm, 5 µm) (p/n 880975-902)
Supercritical fluid:	CO <sub>2</sub>
Modifier:	9:1 ACN/MeOH
Outlet pressure:	150 bar
Flow rate:	2.5 mL/min
Gradient:	0–90 minutes: 2%-10%
Column temperature:	25 °C
Injection volume:	5 µL
Make-up flow:	IPA at 0.6 mL/min
Caloratherm:	60 °C
DAD:	210/4 nm, Ref. 360/100 nm
ELSD:	Evap 30 °C, Neb 30 °C, 1.60 SLM, Gain 1, Smoothing 5 seconds

**Table 2**  
Experimental conditions for reversed phase (C18) separation.

Conditions	
Column:	Chromspher 5 Lipids (4.6 × 250 mm, 5 µm)
Supercritical fluid:	CO <sub>2</sub>
Modifier:	9:1 acetonitrile:isopropanol
Outlet pressure;	150 bar
Flow rate:	1.0 mL/min
Gradient:	3% (10 minutes) – 0.2%/minutes – 20%
Column temperature:	65 °C
Injection volume:	5 µL
Make-up flow:	IPA at 0.3 mL/min
Caloratherm;	60 °C
DAD:	210/4 nm, Ref. 360/100 nm
ELSD:	Evap 30 °C, Neb 30 °C, 1.60 SLM, Gain 1, Smoothing 5 seconds

**Table 3**  
Experimental conditions for silver loaded stationary phase separation.

## Results and discussion

### Reversed phase separation

Figure 1 shows the UV and ELSD chromatograms for the separation of triglycerides in sunflower seed oil. As seen, the ELSD detector is more sensitive than UV detection, giving S/N ratios approximately five times better than in UV detection. In addition, the baseline is more stable than in the UV signal at this low wavelength. (Table 4). Moreover, the response in ELSD is more universal and less dependent on the number of double bonds in the lipid molecule.

In SFC, using a reversed phase C18 column, triglycerides are separated according to the carbon number and the total number of double bonds. By approximation, the elution order is set according to

$$PN = CN - NDB$$

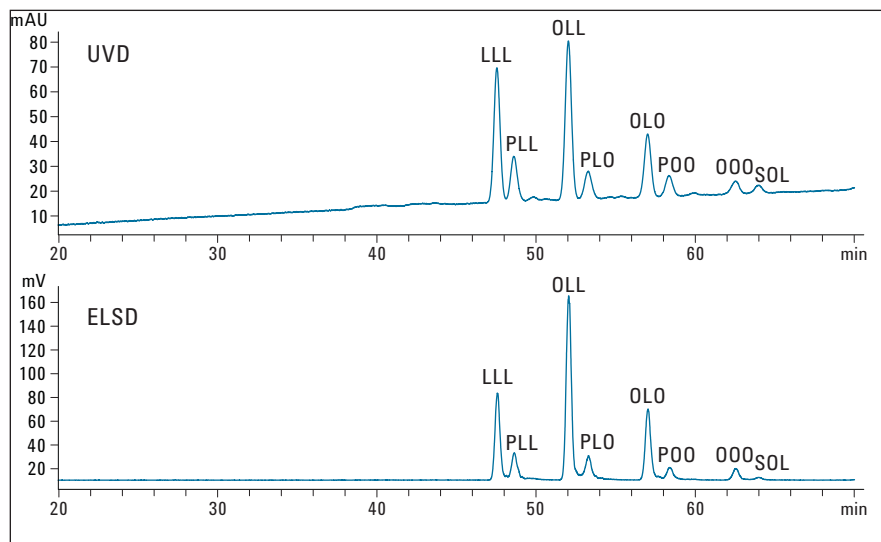
where:

PN = partition number

CN = carbon number (sum of carbons in fatty acid chains)

NDB = sum of number of double bonds

Therefore, the PN for triolein (OOO) is  $(18+18+18)-(1+1+1) = 51$ , and this compound elutes later than OLO with a  $PN = (18+18+18)-(1+2+1) = 50$ . Within a group of triglycerides with an equal PN number, additional separation can be obtained. For example, LLL and PLL (PN = 48), OLL and PLO (PN = 49), and OLO and POO (PN = 50) are separated.

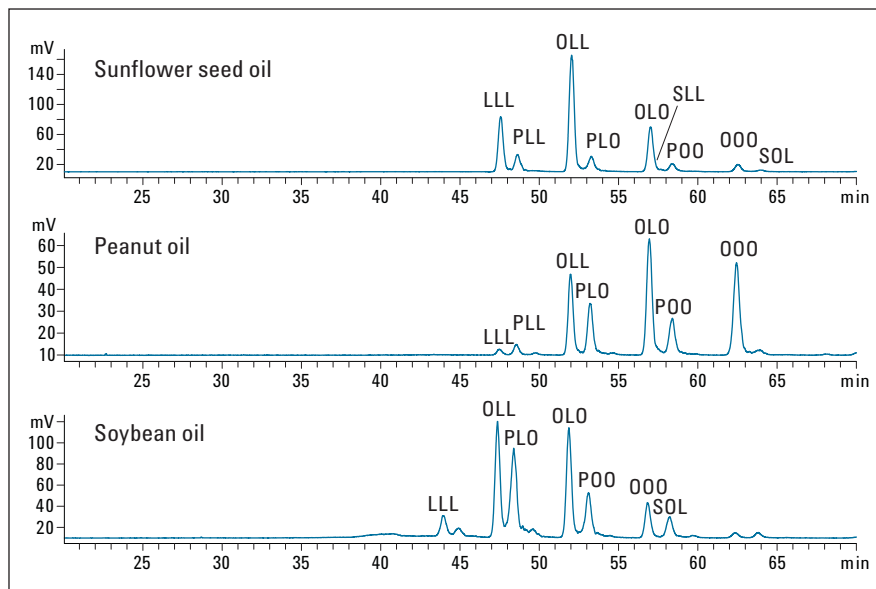


**Figure 1**  
Separation of sunflower seed oil triglycerides with UV detection at 210 nm (top) and ELSD detection (bottom). The separation conditions are listed in Table 2.

	UVD (210 nm)		ELSD	
	Area (mAU*s)	S/N	Area (mV*s)	S/N
LLL	1494	169	1686	1302
PLL	539	56	604	402
OLL	1789	199	3696	2768
PLO	352	34	675	369
OLO	758	79	1572	1070
POO	260	26	342	191
OOO	221	18	295	177
SOL	164	12	71	40

**Table 4**  
Comparison of UVD and ELSD results for sunflower seed oil.

Three different vegetable oil samples were analyzed in another experiment (Figure 2). In all cases, distinct profiles and excellent separation were obtained for all oil types when using SFC with ELSD detection. The detected triglycerides are listed in Table 5 with carbon number, number of double bonds, PN number, and retention times. Elution order clearly corresponds to PN number.



**Figure 2**  
Separation of triglycerides of sunflower seed oil (top), peanut oil (middle), and soybean oil (bottom) on ODS column. Separation conditions are listed in Table 2.

	CN	NDB	PN	ODS column RT (min)	Ag column RT (min)
LLL	54	6	48	47.55	54.88
PLL	52	4	48	48.61	44.72
OLL	54	5	49	52.04	49.94
PLO	52	3	49	53.29	39.92
OLO	54	4	50	57.02	45.19
SLL	54	4	50	57.66	45.19
POO	52	2	50	58.36	34.83
OOO	54	3	51	62.52	40.51

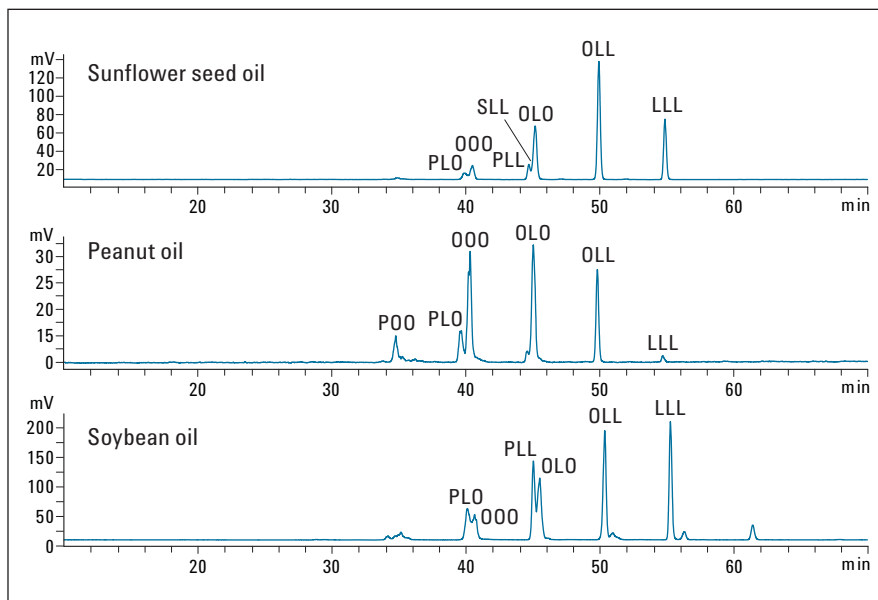
**Table 5**  
Identified triglycerides and retention times on ODS and Ag loaded columns.

## Separation on a silver loaded stationary phase

The separation of the vegetable oils on the silver loaded column is shown in Figure 3. On this column, separation is mainly based on the number of double bonds, resulting in a group type separation of lipids (see also Table 5). Within a group of triglycerides with the same number of double bonds, some partial separation could be observed but to a lower degree as compared with separation on C18 (for example, PLL/OLO). The SFC separation on the silver loaded column is, however, very useful to determine polyunsaturated triglycerides in oils and also be applied for quality control of fish oils.

Analyzing the three vegetable oils on both column types demonstrates a complimentary selectivity, rendering the combination of both SFC methods into an excellent quality control tool of vegetable oils samples.

Retention time and peak area repeatability was tested on both columns for a test mixture containing tripalmitin, triolein and trilinolein. The results are summarized in Table 6. The RSDs (%) on retention times are below 0.2% on ODS and around 1% on the silver loaded column. Peak area repeatability is around 2% on ODS and around 4% on the Chromspher lipid column.



**Figure 3**  
Separation of triglycerides of sunflower seed oil (top), peanut oil (middle), and soybean oil (bottom) on silver loaded column. Separation conditions are listed in Table 3.

		PPP	OOO	LLL
ODS	TR <sub>AVG</sub> (min)	51.72	62.77	47.67
	RSD <sub>TR</sub>	0.16%	0.16%	0.20%
	Area <sub>AVG</sub> (mV*s)	3597	1623	1795
	RSD <sub>Area</sub>	1.53%	1.65%	2.19%
ChromSpher Lipid	TR <sub>AVG</sub> (min)	21.54	40.54	54.75
	RSD <sub>TR</sub>	1.60%	0.93%	0.49%
	Area <sub>AVG</sub> (mV*s)	3080	2509	3146
	RSD <sub>Area</sub>	4.84%	2.61%	4.72%

**Table 6**  
Retention time and peak area repeatability (n=6).

## Conclusion

This Application Note demonstrates the separation of triglycerides in vegetable oil samples using the Agilent 1260 Infinity Analytical SFC System coupled to evaporative light scattering detection (ELSD). The ELSD results were reproducible, and provided enhanced sensitivity compared to UV detection. The separations obtained on octadecyl silicagel (reversed phase) and on a silver loaded stationary phase (ChromSpher Lipid) are complementary and can be applied in quality control of vegetable oils.

## References

1. E. Lesellier and A. Tchaplal, in *Supercritical Fluid Chromatography with Packed Columns, Techniques and Application*, K. Anton and C. Berger (Eds), Chromatographic Science Series Vol 75, Marcel Dekker Inc, New York, pp 195-221, **1998**.
2. M. Lafosse, in *Practical Supercritical Fluid Chromatography and Extraction*, M. Caude and D. Thiebaut (Eds), Harwood Academic Publishers, Amsterdam, pp 201-218, **1999**.
3. P. Sandra, A. Medvedovici, Y. Zhao, F. David, *J. Chromatogr. A.* 974 231, **2002**.
4. M. Dunkle, G. Vanhoenacker, F. David, P. Sandra, M. Vollmer, *The Agilent 1260 Infinity SFC/MS Solution*, Agilent Technologies Publication Number 5990-7972EN, **2011**.

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