Lipids are essential to life as selective barriers for the movement of molecules across the cell membrane. Triglycerides are a type of lipid used by living systems for energy transportation and storage. Foodstuffs such as vegetable oils, olive oil and seed oils contain triglycerides with varying degrees of saturation. Many different types of triglyceride found in these oils give rise to the fact that they posses a unique “fingerprint” that can be used to determine provenance.

SEPARATION OF TRIGLYCERIDES IN SESAME OIL

- **Using the Chromaster system & VWR (ELSD) 90 detector**

A typical lipid in foodstuffs is the triester formed from glycerol and oleic acid, which is the most abundant lipid in olive oil. Oleic acid is a mono unsaturated fatty acid with one Z double bond between C9 and C10 of the C18 chain.

As triglycerides (TAGs) are of low volatility, it makes them unsuitable for analysis by Gas Chromatography. There is also a transesterification derivitisation step but this is not suitable for such complex mixtures.

TAGs are not easy to detect using UV owing to the low wavelength needed (210 nm).
There is an IUPAC HPLC method using refractive index (RI) detection but as RI requires isocratic elution, run times can be long and RI is often sensitive to external factors such as room temperature.

Here we report from the VWR Application Laboratories, a method for the separation and detection of triglycerides using the VWR Hitachi Chromaster and the VWR ELSD 90, the new and highly sensitive low temperature evaporative light scattering detector.

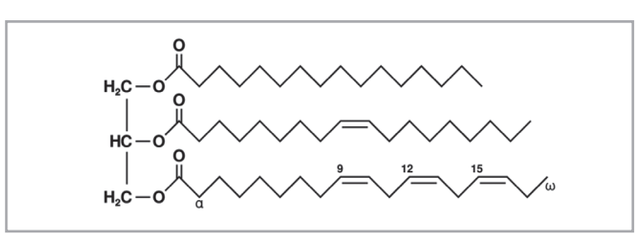

Figure 1: Example of an unsaturated triglyceride. Composed of an ester formed from glygerol with palmitic acid, oleic acid and alpha-linolenic acid. Empirical chemical formula: $C_{55}H_{98}O_6$.

Chromatographic conditions and ordering numbers			
Chromaster package: 903-0591			
5160 Pump including Standard Static Mixer (700 µl), Quaternary Low Pressure Gradient, 6-channel degasser			
5260 Low Carry Over Autosampler			
5310 Oven with Column Management System			
VWR ELSD 90: 903-0267			
Low temperature evaporative light scattering detector with standard nebuliser and driver.			
Eluent solvents: Dichloromethane: 23373.320, acetonitrile: 83639.320, acetone: 20067.320			
Eluent composition	A: Acetone, dichloromethane, acetonitrile (5:15:80 V/V/V)		
	B: Acetone, acetonitrile, dichloromethane (20:20:60 V/V/V)		
Autosampler settings	Syringe speed 2, Cut-Method, lead: 5 µl, rear: 30 µl; syringe: 175 µl		
Gradient mode	Low Frequency Mode (LFM)		
Gradient	Time	A %	B %
	0	100	0
	15	75	25
	25	75	25
	70	0	100
	75	100	0
	90	100	0
Flow rate / run time	0,9 ml/min / 90 min including re-equilibration for the next injection		
Pressure	180 bar		
ELSD conditions	Drift tube temperature: 85 °C, N_2 gas pressure: 3,5 bar, gain 5, filter 4 s, Auto-Zero		
Oven temperature	25 °C		
Column	2x Merck LiChroCart® 250-4, Superspher® 100 RP-18, Cat. No. 1.16056.0001, coupled together in series		
Pre-column	Merck LiChroCart® 4-4, Purospher® RP-18e, Cat. No. 1.50167.0001		
Injection volume	20 µl		
Test solutions	Dilute 50 mg of the sesame oil to 10 ml with a mixture of equal volumes of acetone and dichloromethane		
Reference solutions	Dissolve 80 mg of triolein in a mixture of equal volumes of acetone and dichloromethane and dilute to 50 ml with the same mixture of solvents. Prepare 5 reference solutions by dilution of this solution so as to cover concentrations ranging from the disregard limit (0,5%) to the upper limit for OLL (30%)		

Results

As can be seen from Figure 2, the VWR ELSD 90 is an excellent choice of detector for this application when compared to similar samples detected using refractive index (Figure 3) or UV (Figure 4).

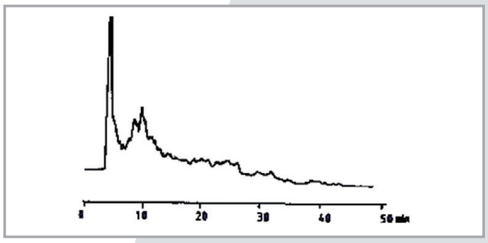

Figure 3: HPLC isocratic separation of triacylglycerols in butter, detection using a Refractive Index Detector (acetone:acetonitrile 65:35).

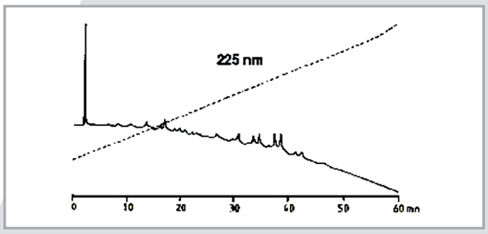

Figure 4: HPLC gradient separation of triacylglycerols in butter, detection using a UV Detector ethanol/ acetonitrile 20% to 100% ethanol in 55 minutes.

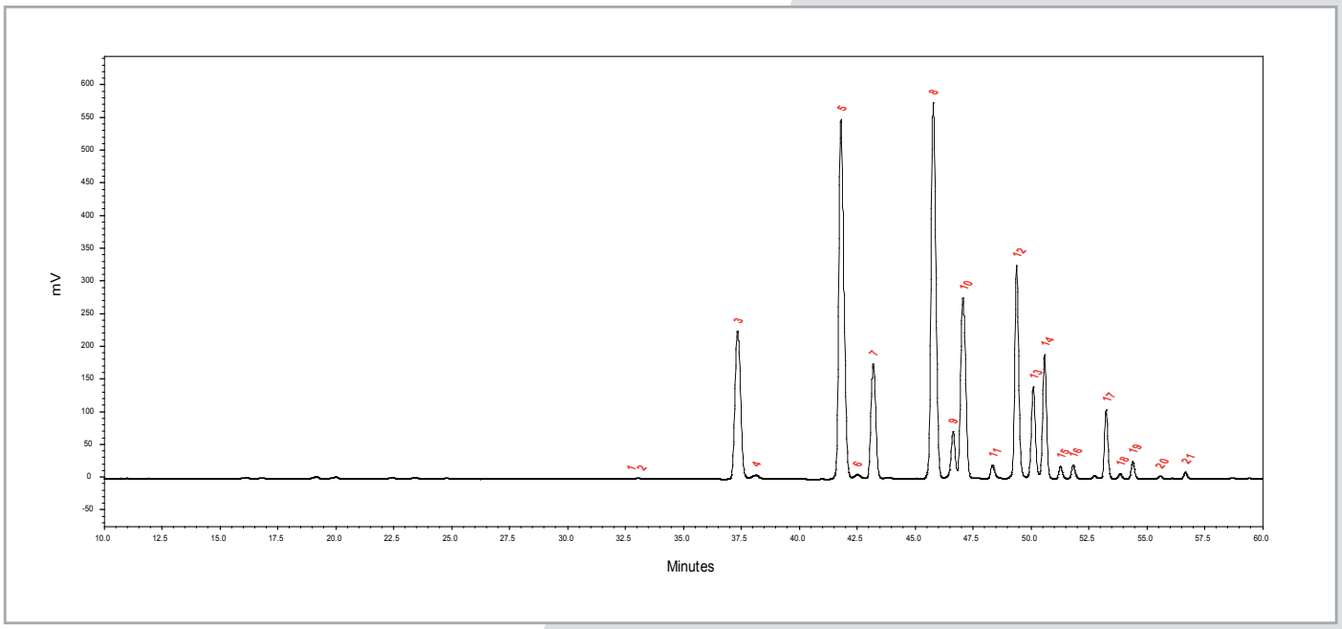

Figure 2: Chromatogram of the separation of triacylglycerols in sesame oil. Owing to the various combinations of the fatty acid chains attached to the ester "head", it is possible to identify the source of the oil using such qualitative chromatographic methods.

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