[TECHNOLOGY BRIEF]

Characterization of Triacylglycerols in Edible Oils Using the ACQUITY UPC^2 System and Mass Spectrometry

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GOAL

To explore Convergence Chromatography (CC) with MS technology for profiling and identifying TAGs.

BACKGROUND

Natural oils and fats are complex mixtures consisting primarily of triacylglycerols (TAGs), which are esters derived from glycerol and three fatty acids (FA). Chromatographic separation of TAGs in oils and fats is a challenging task since a large number of TAG species may exist in the oils and fats.[1] TAG profiles can be used to assess the quality and the authenticity of oil and fat products. High temperature-capillary gas chromatography (HT-CGC) is a common technique for TAG analysis, but the degradation of late-eluting TAGs and the lifetime of the capillary GC column at high temperatures (about 360 °C) cause some concerns. Non-aqueous reversed-phase liquid chromatography (NARP-LC) is the other common technique for TAG analysis. The international reference method for TAGs in vegetable oils is based on the NARP-LC technique.[2] The drawbacks of NARP-LC methods include relatively long run times and the use of toxic solvents.[3] UltraPerformance LC® (UPLC®) can improve the resolution and reduces run times, but toxic organic solvents still have to be used.[4,5]

The ACQUITY UPC^2 System coupled with mass spectrometry provides a simple and powerful approach for profiling and identifying TAGs.

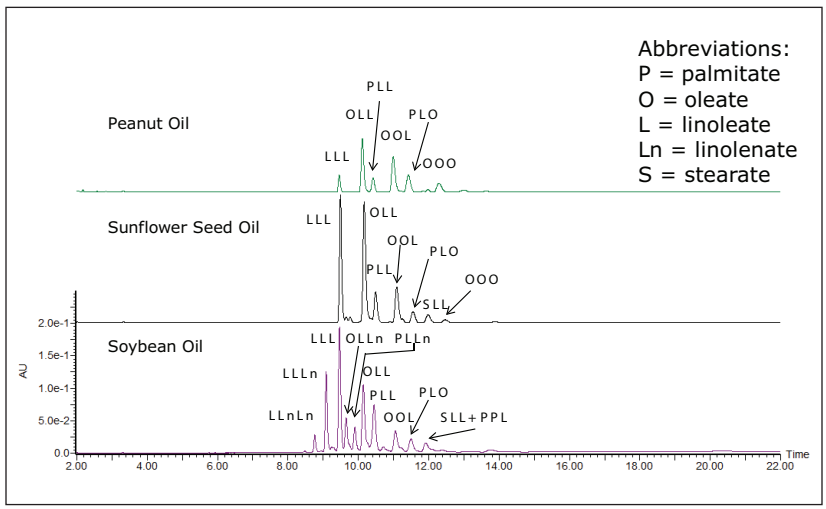

Figure 1. UV chromatograms (210 nm) of TAGs in peanut, sunflower seed, and soybean oils (10 mg/mL in chloroform) on an ACQUITY UPC^2 HSS C18 SB Column (3.0 x 150 mm, 1.8 µm) using UPC.^{2} Flow rate: 1.0 mL/min; column temp.: 20 °C; ABPR: 1500 psi; mobile phase A: CO_2; mobile phase B: ACN; gradient: 3% B for 2 min; then linear gradient to 70% B in 15 min, then hold at 70% B for 5 min. TAG peak assignment was based on accurate mass spectra of the precursor ions and fragment ions obtained on the Xevo G2 QTof Mass Spectrometer.

THE SOLUTION

Waters® UltraPerformance Convergence Chromatography™ (UPC$^{2®}$) leverages the unique properties of compressed CO_2 at or near its supercritical state, such as low viscosity and high diffusivity, as well as packed columns to improve separation efficiency, speed, and selectivity. High separation efficiency, speed, and selectivity are critical for the analysis of complex mixtures of compounds with similar structures, such as TAGs. The low polarity of compressed CO_2 also makes UPC^2 suitable for TAG analysis. Time-of-flight (Tof) mass spectrometers with MS^E technology simultaneously collect the exact-mass of precursor ions at low collision energy and their corresponding fragment ions at high collision energy, which provide essential information for identification and structural elucidation. This is critical as not all TAG standards are commercially available, necessitating the use of a high resolution MS technique like Tof-MS with MS^E for the peak assignment in TAG analysis.

TAGs in peanut, sunflower seed, and soybean oil were separated on an ACQUITY® UPC^2 C18 Column using the ACQUITY UPC^2 System with a gradient elution, shown in Figure 1. All TAGs eluted within 15 minutes and showed baseline separation for all the major TAGs. This is much faster than the HT-CGC and the NARP-LC methods, which usually take 30 to 80 minutes. Compared to UPLC methods,[4,5] UPC^2 has similar run time and resolution, but the column pressure in UPC^2 is much lower, which allows for a higher flow rate or for a longer column to be used. The solvent consumption in UPC^2 is also lower. The Xevo® G2 QTof Mass Spectrometer with an ESI source was coupled to the ACQUITY UPC^2 System. A made-up solvent of methanol with 10-mM ammonium hydroxide was infused into the eluent post-column at 0.2 mL/min to assist with ionization.

TAG peaks were identified using the accurate mass spectra collected by QTof MS with MS^E and Waters TransOmics™ Informatics. Accurate masses of the molecular ions, the fragment ions after the loss of one fatty acid, and the relative abundance of the fragment ions were used in peak assignment. The isotope distribution profile of the molecular ions was also used to confirm the peak assignment.[6] Table 1 lists the accurate masses of molecular ions and fragment ions, the relative abundance of fragment ions, their retention factors, and the identified chemical structures of the TAGs.

In NARP-LC analysis, the retention of TAGs is affected oppositely by two factors: the carbon number (CN) and the number of double bonds of FAs. Increased retention is observed for higher carbon numbers, while retention decreases with increasing number of double bonds. The data from this work suggests that retention of TAGs on an un-endcapped C18 column using UPC^2 with gradient elution follows the same rules. Based on the retention factors and peak assignment data in Table 1, an empirical equation for equivalent carbon number (ECN) is proposed (R=0.99):

$$ECN = CN - 2.55 \times n_o - 1.98 \times n_l - 1.52 \times n_{ln}$$

where n_o, n_l, n_{ln} are the number of double bonds of TAG constituting oleic, linoleic, and linolenic acids, respectively. The origins of the double bonds, either in oleic, linoleic, or linolenic acids, are taken into account to more precisely account for their subtle difference in decreasing the retention time. A correlation coefficient constant of 0.99 for the linear regression between ECN and the retention factor indicates that the retention of TAG under these UPC^2 conditions behaves similarly to that in NARP-LC.

Table 1. The retention factor (k), TAG ammonium adduct precursor exact mass, corresponding DAG fragment mass after one fatty acid neutral loss, and the relative abundance, chemical structure, carbon number and the number of double bonds of major TAGs in three edible oils in the study.

Ret. factor, *k*	Mass of TAG adduct $(TAG+NH_4)^+$	(DAG)+ fragment masses after fatty acid neutral loss and relative abundance[1]			TAG structure[2]	CN	Double bonds
		1	2	3			
5.38	892.7387	597.4878(67)	595.4721(33)		18:2/18:3/18:3	54	8
5.64	894.7545	597.4878(67)	599.5034(33)		18:2/18:2/18:3	54	7
5.87	896.7695	599.5009(100)			18:2/18:2/18:2	54	6
6.00	896.7702	599.5034(33)	597.4878(33)	601.5191(33)	18:1/18:2/18:3	54	6
6.16	870.7545	597.4878(33)	575.5034(33)	573.4878(33)	16:0/18:2/18:3	52	5
6.34	898.7825	601.5186(67)	599.5009(33)		18:1/18:2/18:2	54	5
6.52	872.7662	575.5038(67)	599.5059(33)		16:0/18:2/18:2	52	4
6.95	900.7977	601.5186(67)	603.5347(33)		18:1/18:1/18:2	54	4
7.23	874.7883	601.5186(33)	577.5201(33)	575.5038(33)	16:0/18:1/18:2	52	3
7.52	900.7977	603.5347(67)	599.5059(33)		18:0/18:2/18:2	54	4
7.64	848.7722	575.5038(67)	551.5060(33)		16:0/16:0/18:2	50	2
7.75	902.8274	603.5347(100)			18:1/18:1/18:1	54	3

1: Expressed as m/z (abundance%).

2: The sn-1, sn-2, and sn-3 acyl chain position assignments are arbitrary.

SUMMARY

Baseline resolution of major TAGs in edible oils (peanut, sunflower seed, and soybean oil) was achieved using UPC^2 on an un-endcapped C18 column within 15 minutes, which was faster than that in the HT-CGC and the NARP-LC methods. Chemical structures of TAGs were identified using accurate mass spectra obtained from QTof MS with MS^E. The retention of TAGs under UPC^2 conditions on the un-endcapped C18 column followed the same behavior as in NARP-LC separation. This technology brief demonstrates that UPC^2 coupled with QTof MS with MS^E is a simple and powerful technique for the analysis of TAGs.

References

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