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Fatty Acid Profiling by UPC²-MS

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Waters ACQUITY UPC² System provides highly efficient separation of the positional and trans/cis geometrical fatty acid isomers.

GOAL

Demonstrate the capability of the ACQUITY® UPC²®–MS system for fatty acid profiling of food.

BACKGROUND

The development of a fast and simple analytical method for the routine simultaneous identification and quantification of a variety of fatty acids (FA) is desirable for use in various fields. The profiling of fatty acids has mainly been carried out by Gas Chromatography (GC). However, the fatty acids need to be converted to their esters. In addition, the non-volatility of long-chain fatty acid esters and the thermally labile property of unsaturated fatty acids can further complicate GC analysis. Reversed-Phase Liquid Chromatography (RPLC) has been widely employed for fatty acid determination. RPLC based methods do not require derivatization, and there is no concern about the compound's volatility, nor their high temperature stability. However, RPLC is less efficient in fatty acid separation than GC is.

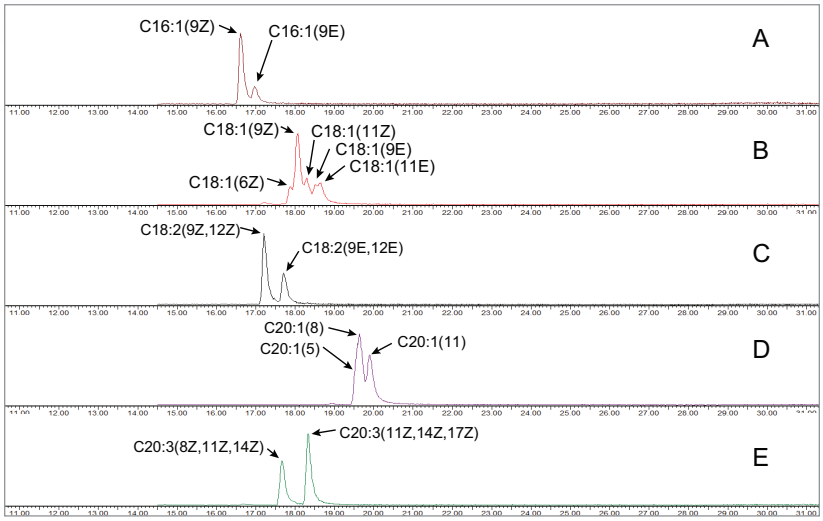

Figure 1. Examples of UPC²-MS chromatograms of fatty acid isomers: A. C16:1 9Z, and 9E; B. C18:1 6Z, 9Z, 11Z, 9E, 11E; C. C18:2(9Z,12Z) and C18:2(9E,12E); D. C20:1 Δ5, Δ8, Δ11 isomers; and E. C20:3(8Z,11Z,14Z) and C20:3(11Z,14Z,17Z). These extracted ion chromatograms (XIC) were achieved on two ACQUITY UPC² HSS C_{18} SB Columns (3.0 x 150 mm, 1.8 µm) connected in series; Column temp.: 10 °C; Co-solvent (mobile phase B): MeOH/AcN(50/50) with 1% formic acid; Flow rate: 0.70 mL/min; Gradient: Keep 1% B for 3 min., ramp to 6% B in 17 min., then keep at 6% B for 2 min., ramp to 20% B in 1 min., and hold at 20% B for 5 min., return to 1% B and condition the column for 7 min.; Injection volume: 0.5 µL. ABPR: 1500 psi. The peak IDs were verified with the individual standards.

Waters® UltraPerformance Convergence Chromatography™ (UPC²) is the next-generation supercritical fluid chromatography (SFC) system that provides excellent separation efficiency and speed in a wide range of application areas, including edible oils, acylglycerols, and short-chain fatty acids. In this technology brief we demonstrate the capability of UPC²-MS for the separation of positional and geometrical fatty acid isomers, and its feasibility for fatty acid profiling.

THE SOLUTION

The ACQUITY UPC² System offers excellent separation efficiency for fatty acids. The separation performance of UPC² for the positional and geometrical analysis of fatty acid isomers (Figure 1) is comparable to GC, and it is much better than that in RPLC. Under UPC² conditions, the fatty acids were separated based on their chain length, degree of saturation, and geometrical configuration, as shown in Figure 2. The retention time (RT) increased with increasing chain length and decreasing number of double bonds. The trans isomers were eluted after the corresponding cis isomers. In addition, the closer the double bond to the carboxyl group, the more reduction in the RT (Figure 1B, 1D, and 1E).

When coupled with tandem quadrupole mass spectrometry (Waters Xevo® TQ-S) , UPC² provided a fast and relatively simple method for the determination of the fatty acid profile. The total run time, including a seven minute column equilibration between injections, was 35 minutes, which is about half of a typical GC analysis run time. There was no need to convert the fatty acids to their esters. This method has been successfully applied to the determination of free fatty acids in fat extracts from food, as shown in Figure 3.

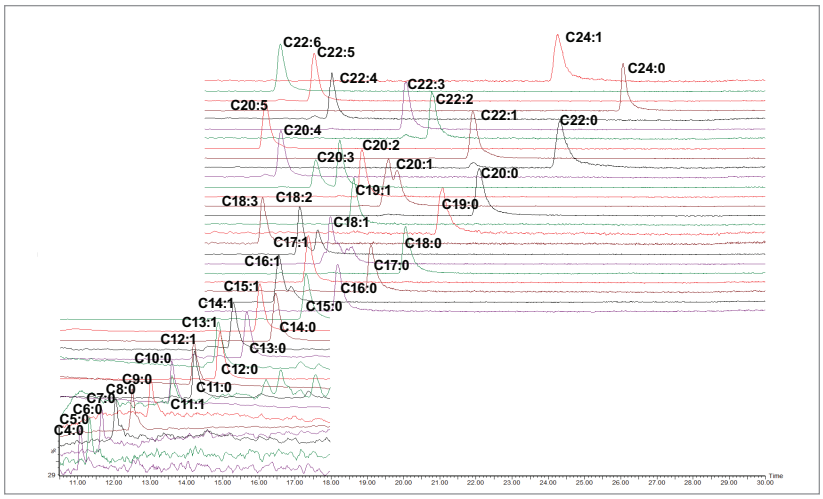

Figure 2. Overlay of chromatograms of 51 fatty acid compounds in a standard mix (GLC-463 fatty acid mix from Nu-Chek Prep, Inc., Elysian, MS). The chromatograms of fatty acids from C4 to C15 were SIR chromatograms, and the chromatograms for C16 to C24 were XIC from MS spectrum. Peak labels are shown in the chromatograms.

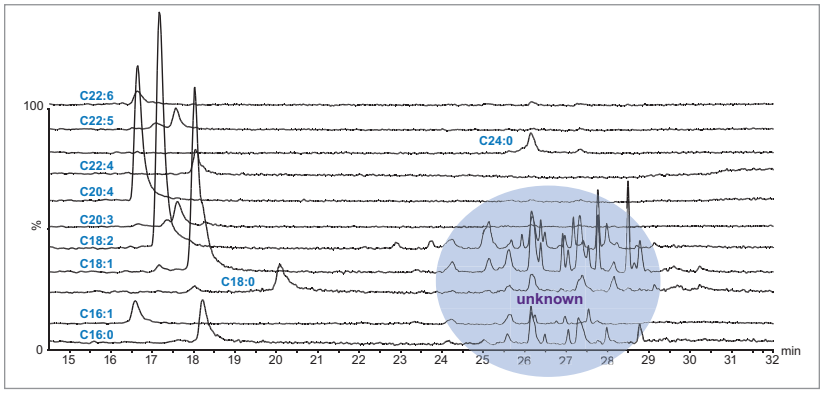

Figure 3. Selected XIC of the free fatty acids in the sample. The peaks were identified by their m/z values and the reference retention times of the corresponding fatty acid standards. The shaded area were the unknowns, which were believed to have come from the sample matrix. The identified peaks were quantified with the corresponding calibration curves (Results not shown).

SUMMARY

Waters ACQUITY UPC² System provides excellent separation for the positional and trans/cis geometrical fatty acid isomers. When coupled to the Xevo TQ-S Mass Spectrometer, this UPC²-MS system provides a fast and simple approach for fatty acid profile determination. The run time is about half that of the typical GC method, and there is no need for derivatization. The elimination of the derivatization step brings the additional benefits of reduced chemical waste and simplified sample preparation. The UPC²-MS system provides an alternative approach for fatty acid profiling, especially for samples that contains thermal labile fatty acids and long chain fatty acids.

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