APPLICATION BRIEF

Chemical/Industrial

Determination of Fatty Acids in Biodiesel Fuel by UHPLC/SQ MS

Due to the depletion of fossil fuels, biodiesel fuel is gaining more importance and becoming an appealing alternative. Since biodiesel fuel is produced from plant oils and animal fats, fatty acids are a primary component of the fuel. The amount of fatty acids affects the efficiency of the esterification process which is necessary for the fuel to be usable. The presence of fatty acids in biodiesel fuel that have not been trans-esterified can cause engine degradation and produce hazardous emissions.

Here we present a method that is fast and sensitive for the monitoring of individual fatty acids in a biodiesel fuel sample using ultra high performance liquid chromatography (UHPLC) and a single quadrupole mass spectrometer (SQ MS). Other lipid species found in the oils such as triglycerides and phospholipids can also be monitored using this method without esterification and minimal sample preparation. Quick and sensitive analysis of fatty acids in biodiesel using UHPLC/SQ MS followed by an intuitive and easy-to-use data analysis software package provides a powerful and robust method for monitoring biodiesel quality.

Experimental Conditions

Target Analytes: Palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoelic acid (C18:2), linolenic acid (C18:3), arachidic acid (C20:0), and gadoleic acid (C20:1)

Sample Preparation Conditions

50 µL of biodiesel fuel was extracted with 950 µL of 2:1 dichloromethane:methanol solution. The sample was vortexed for 10 sec and then centrifuged for 10 min at 12000 RPM. The sample was diluted 1:100 with 2:1 dichloromethane:methanol solution prior to injection.

Liquid Chromatography Conditions

Pump Type:	PerkinElmer® Flexar™ FX-15
Column:	PerkinElmer Brownlee™ HRes C18 column (2.1 mm x 100 mm, 1.9 µm)
Mobile Phase:	A: 40/60 water/acetonitrile with 12 mM ammonium acetate B: 90/10 isopropanol/acetonitrile with 12 mM ammonium acetate
Flow Rate:	0.5 mL/min
Column Temp:	55 °C
Injection Volume:	1 µL

Gradient:

Time (min)	%A	%B
0	40	60
10	0	100
1	0	100
1	40	60

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Mass Spectrometer Conditions

Ionization:	Ultraspray™ ESI – Negative mode for fatty acids Ultraspray™ ESI – Positive mode for triglycerides and phospholipids
Scan Range:	100-1000 m/z
Scan Rate:	2500 u/sec
Selected Ion Monitoring (SIM) Mode:	Reported in Figure 1
Capillary Exit Voltage:	-100 V for negative Scan data; 100 V for positive Scan data; 100V for SIM data

Results

The total ion chromatogram shows that there are many peaks found in the biofuel oil sample shown in Figure 2. Extracted ion chromatograms allow for the identification of fatty acids of interest. After identification of the fatty acid retention times in Scan mode, Selected Ion Monitoring (SIM) can be used to monitor the fatty acids for quantitation at lower concentrations shown in Figure 3. The increased saturation of the fatty acids correlates with the increased retention times. ESI positive Full Scan mode acquisition shows additional lipid species such as triglycerides that can be extracted from the TIC (Figure 4). Future experiments will include quantitation of fatty acids in biodiesel fuel.

Figure 1. $[M+H]^-$ of each analyte in SIM mode.

Analyte	SIM m/z
C16:0	255.1
C16:1	253.1
C18:0	283.1
C18:1	281.1
C18:2	279.1
C18:3	277.1
C20:0	311.1
C20:1	309.1

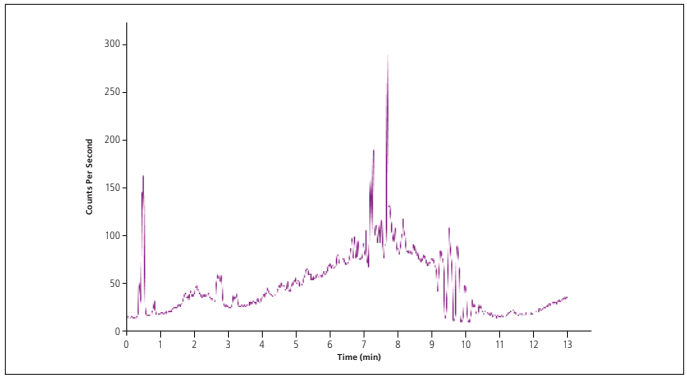

Figure 2. ESI negative mode chromatogram of the oil sample.

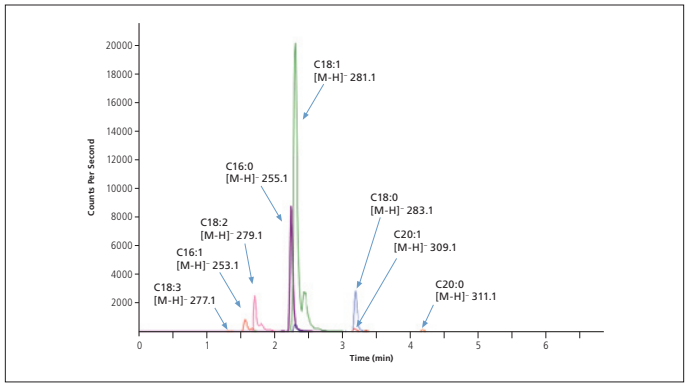

Figure 3. Overlaid chromatograms of $[M-H]^-$ ions for 8 individual fatty acids in ESI negative SIM mode.

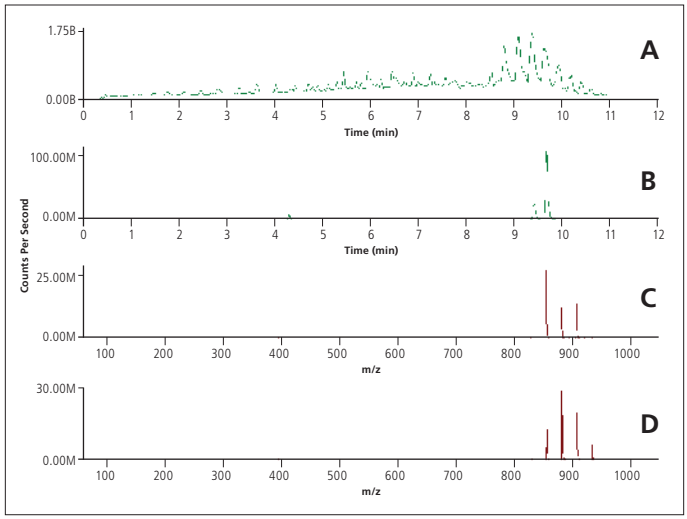

Figure 4. A) ESI positive mode Full Scan chromatogram of biofuel extract.
B) Extracted ion chromatogram of 850.8 m/z.
C) Average mass spectrum of a triglyceride under peak 1.
D) Average mass spectrum of a triglyceride under peak 2.

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