Application Note

No. 220055S-E

Supercritical Fluid Chromatography of Fatty Acid Methyl Esters and Triglycerides in Biodiesel Fuel with Charged Aerosol Detector

Introduction

The main constituents of biodiesel fuel fatty acid methyl esters, which are obtained by trans-esterification of triglycerides contained in vegetable oil. Thus, determination of fatty acid methyl esters and triglycerides is essential to characterization of biodiesel fuel. Carbon dioxide exhausted from the combustion of biodiesel fuel is reabsorbed by plants through photosynthesis, offsetting the amount of carbon dioxide emission.

Refractive index detectors (RIDs) have been used to detect fatty acid methyl esters. However, a refractive index detector offers only a limited sensitivity, and is not compatible with gradient elution. In addition, organic solvents used in HPLC are not environmentally friendly.

Supercritical fluid chromatography (SFC) is known as green chromatography, reducing organic solvent disposal. Charged aerosol detectors (CADs) can be applied to supercritical fluid chromatography (SFC), allowing a gradient elution, and can expect an increase in the detection of weak-ultraviolet-absorbing components such as fatty acid methyl esters and triglycerides.

We applied supercritical fluid chromatography (SFC) coupled with CAD to analysis of biodiesel fuel.

Experimental

JASCO HPLC 2000 series modular components were used for the measurement system. The system consisted of a PU-2080-CO_2 CO_2 delivery pump, a PU-2080 pump (3 sets), a CO-2060 column oven, an MX-2080 mixer (1.5 mL), an MD-2010 photodiode array detector, a BP-2080-M backpressure regulator, an AS-2059-SF autosampler, and ChromNAV chromatography data system. The charged aerosol detector was Corona CAD Plus (ESA).

The separation column was a SFCpak SIL-5 (4.6 mmID x 250 mmL, 5 µm). The flow rates of CO_2, modifier, and make-up solvent were 3.0 mL/min, 0.4 mL/min or 1.0 mL/min, and 0.5 mL/min, respectively. Modifiers were prepared with mixtures of hexane and IPA, (98/2) and (80/20). These modifiers were delivered stepwise as follows: 0.0 – 1.0 min, (98/2), 0.4 mL/min; 1.0 – 3.0 min, (80/20), 0.4 mL/min; 3.0 – 4.0 min, (80/20), 1.0 mL/min.

The column temperature was set to 40°C, and the back pressure to 20MPa. The stock solution was prepared by dissolving methyl stearate (1.0 g) and trilinolein (0.01 g) in diesel fuel to make the volume up to 10 mL. The standard solutions were then prepared by diluting the stock solution twice, five times, ten times, and one hundred times using the diesel fuel.

Results and Discussion

The schematic diagram of this system is shown in Figure 1. The effluent from the column was split before the regulator. Then, the split effluent was mixed with make-up solvent (methanol) before the detector.

Figure 2 shows an SFC chromatogram obtained by injecting 5 µL of the standard solutions with different concentrations. Ten-time consecutive injections of the standard solution (twice-diluted stock solution) indicated that the relative standard deviations (RSDs) of retention times for methyl stearate and trilinolein were 0.50% and 0.28%, respectively. RSDs of peak areas for methyl stearate and trilinolein were 3.7% and 2.8%, respectively.

In order to examine the quantitative precision, 5µL of the stock solution and the standard solutions with various concentrations were injected. Obtained peak areas were plotted against the concentrations of the standard solutions. The calibration curve for methyl stearate exhibited a quadratic as follows:

$$y = -2.0432x^2 + 5779.9x + 57600 \ (r^2 = 0.9992) \quad (1)$$

where y indicates peak area (µVsec), x concentration (µg/10 mL), and r coefficient of correlation.
As for trilinolein,

$$y = -1682.5x^2 + 51426x + 15449 \ (r^2 = 0.9988) \quad (2)$$

Figure 3 shows an SFC chromatogram of biodiesel fuel. Near the retention time of methyl stearate appeared several peaks, which corresponded to fatty acid methyl esters, and near the retention time of trilinolein eluted several peaks, which corresponded to triglycerides.

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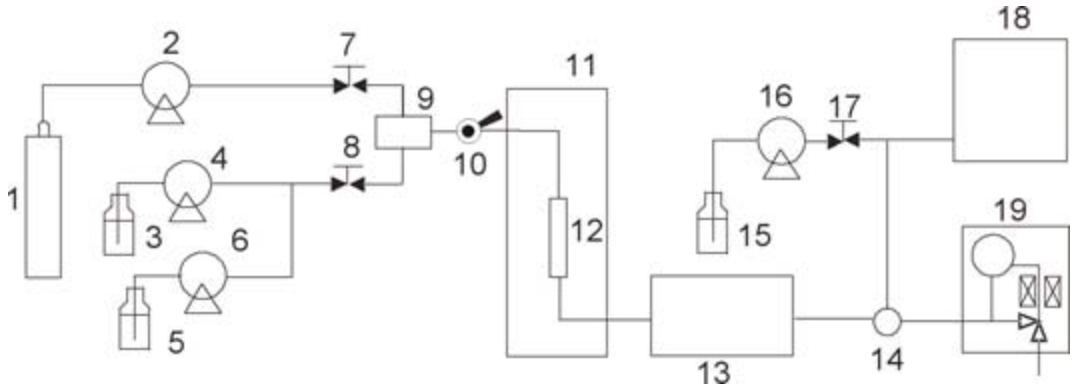

Figure 1 Schematic diagram of the system.
System configuration: 1 = carbon dioxide bottle, 2 = liquefied carbon dioxide delivery pump, 3 = modifier_1, 4 = modifier delivery pump_1, 5 = modifier, 6 = modifier delivery pump_2, 7 = stop valve_1, 8 = stop valve_2, 9 = mixer (internal volume = 1.5mL), 10 = autosampler, 11 = column oven, 12 = separation column, 13 = photodiode array detector, 14 = splitter, 15 = make-up solvent, 16 = solvent delivery pump_3, 17 = stop valve_3, 18 = charged aerosol detector, 19 = automatic back pressure regulator.

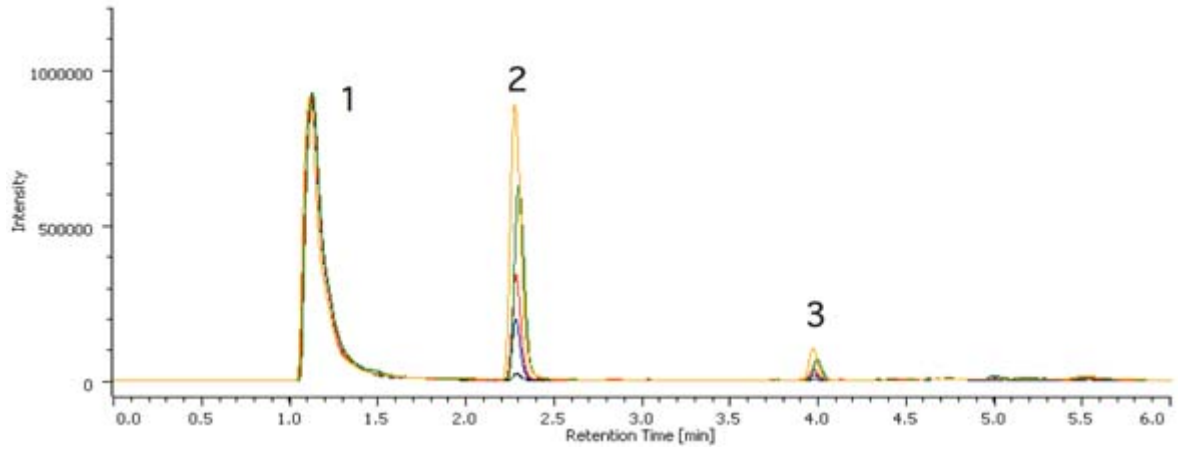

Figure 2 SFC chromatogram of standard mixtures with different concentrations
Peaks: 1=diesel oil components, 2=methyl stearate, 3= trilinolein
SFC conditions: column = SFCpak SIL-5 (4.6 mmID x 250 mm, 5 µm), CO_2 flow rate = 3.0 mL/min, modifier = 0.0–1.0 min, hexane/IPA (98/2), 0.4 mL/min; 1.0 – 3.0 min, hexane/IPA (80/20), 0.4 mL/min; 3.0 – 4.0 min, hexane/IPA (80/20), 1.0 mL/min; column temperature=40°C, pressure=20 MPa, make-up solvent=methanol, make-up solvent flow rate=0.5 mL/min, injection volume=5 µL

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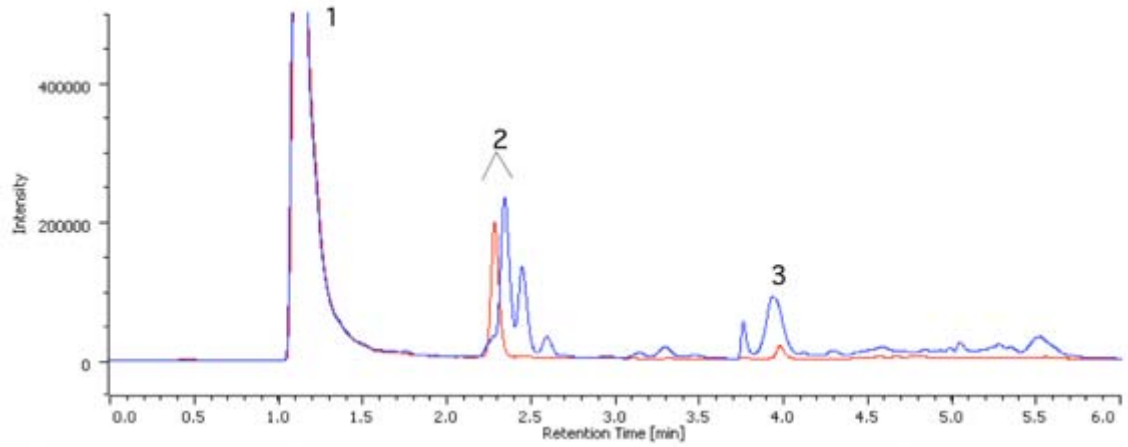

Figure 3 SFC chromatogram of biodiesel fuel. Red line: standard solution;Blue line: biodiesel fuel.
Peaks: 1=diesel oil components, 2= fatty acid methyl esters, 3= triglycerides

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