

# TharSFC

## Fast Separation of FFA, FAME, and Glycerol for Biodiesel Analysis by Supercritical Fluid Chromatography

Jacquelyn Cole, Jennifer Lefler, and Rui Chen, Thar Instruments, Inc.

The alternative fuel is increasing in popularity owing to various technical and economic factors, especially the upsurge in petroleum prices and the implementation of financial incentives for its use. According to the National Biodiesel Board, the sales volume of the alternative fuel in the United States has risen from 50,000 gallons in 1999 to 75 million gallons in 2005 (1).

Biodiesel holds a prominent position among the alternative fuels. Defined as the monoalkyl esters of vegetable oils or animal fats, biodiesel is obtained by reacting the parent vegetable oil or fat with an alcohol, most commonly methanol, in the presence of a catalyst, usually a strong base such as sodium or potassium hydroxide. The resulting product can contain not only the desired alkyl ester, but also un-reacted starting material (TAG), residual alcohol, residual catalyst, by-product glycerol and free fatty acids (FFA), intermediates monoacylglycerols (MAG) and diacylglycerols (DAG) (2). Rapid, reliable and precise determination of the contaminants in biodiesel is of great importance for both process monitoring and product quality control.

Gas chromatography (GC) and high performance liquid chromatography (HPLC) are common analytical techniques for the analysis of biodiesel. GC analysis, considered "the official method", as outlined by ASTM D6584, typically involves a derivatization step that is time- and reagent-consuming (2,3). HPLC analysis, although effectively eliminating the derivatization step, still averages more than 20 minutes per run (2,4); with the exception of one report by Lee et al (5), in which an UPLC was used and the overall analysis time was reduced to 12 min.

Considered by many the "greener" alternative to HPLC, supercritical fluid chromatography (SFC) has experienced striking resurgence and continuously increasing acceptance by the chromatographic society in the past decade, particularly in pharmaceutical and chemical laboratories. The intrinsic low viscosity and high diffusivity of supercritical CO<sub>2</sub> has rendered SFC higher separation speeds and efficiency than traditional HPLC. One of the most important SFC application areas is in fossil fuel analysis, manifested by the two widely used ASTM methods, D5186 and D6550. Recently, Diehl and DiSanzo from Exxon Mobile reported their study on modifying ASTM D5186 for biodiesel analysis using SFC (6).

We present herein our preliminary study on using SFC for fast biodiesel analysis. We prove in principle that fatty acid methyl esters (FAMES), FFAs, and glycerol can be readily separated by using SFC in less than 5 minutes; and hence, SFC holds great potential to become the choice of chromatographic technique for biodiesel analysis. A more comprehensive study, including method optimization and quantification, will be published shortly.

**Table I: Key parameters of the detectors**

Waters 2998 PDA	200–240 nm at a resolution of 1.2 nm
Waters 2420 ELSD	Nebulizer temperature: 50 °C Tube temperature: programmed to 70 °C with a setting of 50 psi and gain set to 1.
Waters 3100 MS	Corona: 20 uA Source Temp: 150 °C Cone: 30 V Probe Temp: 350 °C Extractor: 5 V Desolvation Gas: 350 L/hr Cone Gas: 70 L/hr

### Experimental

**Materials:** All chemicals were used as purchased. Glycerol was purchased from Thermo Fisher Scientific. FAME mix GLC-20, including methyl palmate, methyl oleate, methyl stearate, methyl linoleate, and methyl arachidate was purchased from Supelco. Palmitic acid and oleic acid were from Sigma Aldrich. All chemicals were dissolved in methanol with a total concentration of 60 mg/ml.

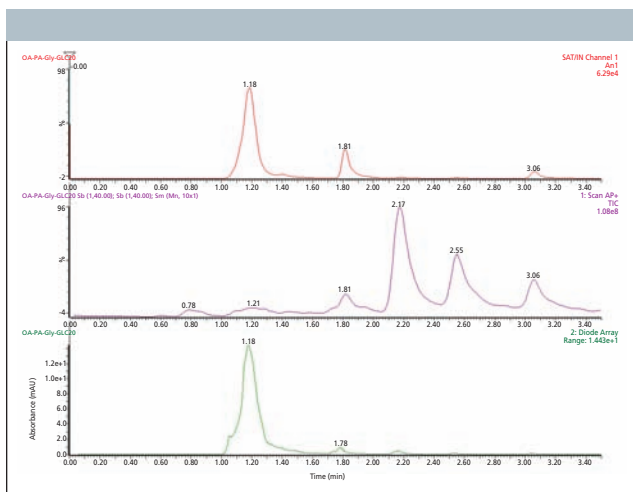
**Instrumentation:** All experiments were carried out using a Thar SFC-MS-UV-ELSD system from Thar Instruments (Pittsburgh, PA, USA). The system consists of a Thar analytical SFC system, a Waters 2998 PDA, a Waters 3100 mass spectrometer, and a Waters 2420 ELSD. Key operational parameters are listed in Table I. A detailed configuration can be found elsewhere (7).

**Chromatography:** All experiments were carried out using a Luna C-18(2) column (4.6x 250 mm, 5 μm) from Phenomenex (Torrance, CA, USA). An isocratic method using 40% acetonitrile at a flow rate of 3.0 mL/min was used for all experiments. Inlet pressure was set at 100 bar and oven temperature was 30 °C.

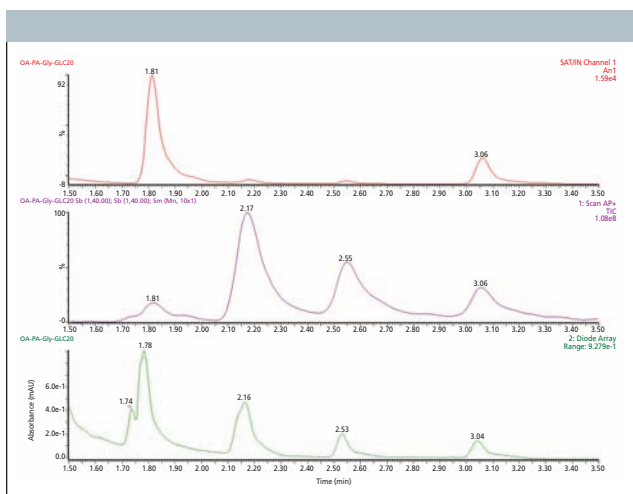
### Results and Discussion

The utility of SFC methodology for biodiesel analysis is illustrated in Figure 1 and 2 with an overall analysis time of 3.5 minutes. The retention times of all the components in the mixture are also listed in Table II.

It is interesting to note that the three main classes of compounds in biodiesel, namely, glycerol, FFA, and FAME, are separated in group, with glycerol being the first to elute off the column and FAMES being the last. In biodiesel production, information on the relative amount of FAME, FFA, and glycerol is sufficient for critical process decision-making. Unlike the group separation in ASTM D5186 for fossil fuel analysis, the group separation of aforementioned compounds by SFC does not involve any valve-switching scheme and is achieved with a



**Figure 1:** Chromatogram of FAMES, FFA and Glycerol mixture. From top to bottom are ELSD, MS (TIC) and UV trace, respectively.



**Figure 2:** Expanded chromatogram of FAMES, FFA and Glycerol mixture (1.5-3.5 min). From top to bottom are ELSD, MS (TIC) and UV trace, respectively.

C18 column in 3.5 minutes; and such separation can provide a good foundation for further quantification. It is also noted (results not shown) that elution order of these classes of compounds can be changed with the change in modifier percentage.

Figure 3 shows the extracted ion chromatograms (EICs) of all the components in the mixture except glycerol. The use of mass spectrometric detectors eliminates any ambiguities on the identity of the eluting components.

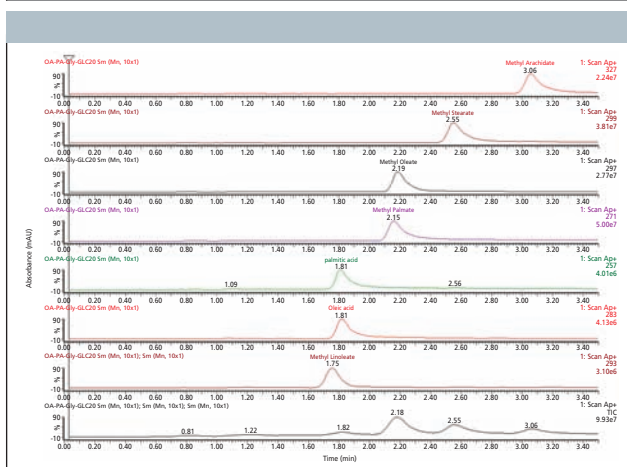
Future work will include further method optimization exploring different combinations of solvent and column, and quantification of the compounds of interest.

## Conclusion

A fast SFC method for the group separation of glycerol, FFAs, and FAMES, the main target compounds for biodiesel analysis, has been developed. The overall analysis time was 3.5 minutes, representing a 3- and 5- fold increase in throughput compared to UPLC and HPLC, respectively. The compatibility of multiple LC-type detectors, including PDA, MS, and ELSD, with SFC provides a facile means for biodiesel process monitoring and quality control.

**Table II: Retention time of the identified components in the mixture**

Component Name	Retention Time (min)
Glycerol	1.18
Methyl Linoleate	1.73
Palmitic Acid	1.81
Oleic Acid	1.81
Methyl Palmate	2.15
Methyl Oleate	2.19
Methyl Stearate	2.55
Methyl Arachidate	3.06



**Figure 3:** Extracted ion chromatograms (EICs) of all the components in the mixture except glycerol.

## References

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**Thar Instruments, Inc.**

575 Epsilon Drive, Suite 100  
Pittsburgh, PA 15238  
tel. (412) 967-5665, fax 412 967-9446  
info@tharsfc.com, www.tharsfc.com