Determination of Residual Acylglycerols in Biodiesel

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Introduction

Many methods have been proposed to determine acylglycerols in biodiesel samples. This analysis is commonly performed by gas chromatography/flame ionization detection (GC/FID) methods such as ASTM D6584 and EN14105. However, GC methods often require sample derivatization, and high inlet temperatures can cause thermal degradation and underestimation of the sample amount. High-performance liquid chromatography (HPLC) analysis with a universal detector, such as charged aerosol detection (CAD), is emerging as the preferred method for biodiesel analysis. This technique is well suited to determine components with low volatility relative to the mobile phase used for separation.

Previous work has demonstrated that the ASTM GC method and HPLC methods can be statistically equivalent.[1] HPLC also permits direct analysis of biodisesel samples with just a simple dilution, precluding the derivatization step that is required for GC analysis of nonvolatile samples. Additionally, by using a small particle-size column, resolution can be improved and run times shortened.

This study demonstrates use of a rapid separation liquid chromatography (RSLC) method for analysis of acylglycerols in biodiesel fuel in under 10 min. This method avoids exceptionally toxic or environmentally damaging solvents, such as propionitrile and dichloromethane, which have been used for acylglycerol determination.[2]

Figure 1 shows the separation of a B99 biodiesel sample produced from used fryer oil (B) and a mixture of acylglycerol standards (A). The triacylglycerols elute later than the fuel methyl esters and are well separated and easily detected using the Thermo Scientific™ Acclaim™ RSLC 120 C18 column and the Thermo Scientific™ Dionex™ Corona™ *ultra*™ Charged Aerosol Detector. The wide dynamic range of the Corona *ultra* Charged Aerosol Detector permits the detection of low-abundance oleoylglycerols in the presence of highly concentrated components in a single analytical run. The use of UHPLC

SPE Column:	Acclaim RSLC 120 C18 2.2 µm, 2.1 × 150 mm
Eluent A:	Acetonitrile
Eluent B:	Ethyl Acetate
Gradient:	0–15% B in 0.5 min, 15–65% B in 3.5 min 65–80% B in 5 min, 80% B for 1 min 2 min equilibration before injection at 0% B
Flow Rate:	0.5 mL/min
Temperature:	15 °C
Inj. Volume	3 µL
Detection:	CAD Nebulizer = 15 °C, Filter = none, Nitrogen Pressure = 35 psi
Sample:	A) Acylglycerol standards B) B99 biodiesel, 5 mg/mL in ethyl acetate

Peaks:		A	B
	1. Methyl Linolenate		
	2. 1-Oleoyl-rac-Glycerol	5.0	5.6 µg/mL
	3. Methyl Linoleate		
	4. Methyl Oleate		
	5. Methyl Palmitate		
	6. Methyl Stearate		
	7. Dilinoleoyl-rac-Glycerol	5.0	<5.0
	8. Methyl Arachidate		
	9. 1,3 and 1,2-Dioleoyl Glycerols	5.0	ND
	10. Trioleoyl Glycerol	5.0	<5.0

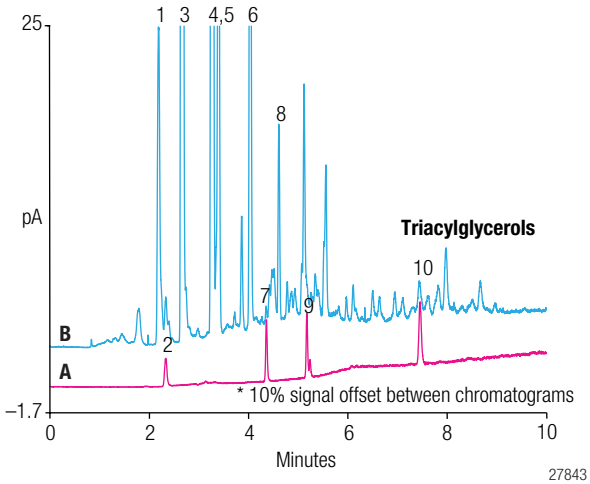

Figure 1. Separation of acylglycerols on the Acclaim RSLC 120 C18 column.

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methods on the RSLC system with CAD detection has the sensitivity to determine acylglycerols in biodiesel samples at and below the 0.24% and 0.25% total glycerol limits in ASTM D6751 and EN 14214, respectively. This method provides a rapid and sensitive acylglycerols determinination in biofuels with minimal sample preparation.

Equipment

- Thermo Scientific™ Dionex™ UltiMate™ RSLC system with an HPG-3400RS pump
- Corona *ultra* Charged Aerosol Detector
- UltiMate 3000 Thermostatted Analytical Autosampler

Conditions

See Figure 1 for chromatography conditions.

Sample Preparation

Prepare biodiesel samples by dilution to 5 mg/mL in ethyl acetate prior to analysis.

References

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2. Buchgraber, M.; Ulberth, F.; Emons, H.; Anklam, E. Triacylglycerol Profiling by Using Chromatographic Techniques. *Eur. J. Lipid Sci. Technol.* **2004,** *106,* 621.

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