A Single Method for the Direct Determination of Total Glycerols in All Biodiesels Using Liquid Chromatography and Charged Aerosol Detection

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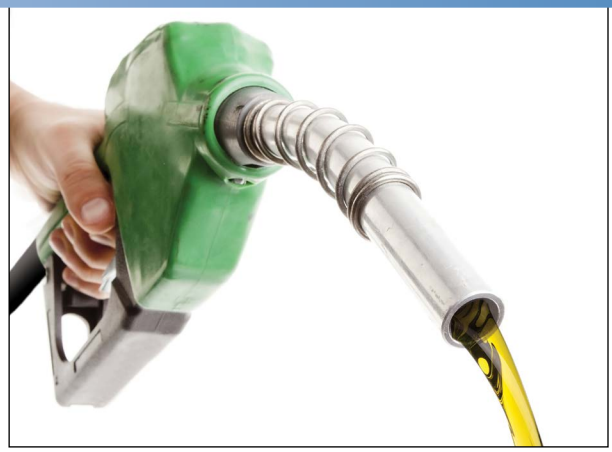
Application Note 1049

Key Words
ASTM method D6584, biodiesel, charged aerosol detection, FAMEs, glycerols, HPLC

Goal
To develop an HPLC method for the determination of acylated and free glycerols in biodiesel fuel, including in-process, finished product, and petroleum-mixed samples.



Introduction
Biodiesel provides a clean and renewable liquid fuel that can be used in current diesel engines and oil burners without significant modifications. Natural oils, such as virgin and waste cooking oils and algal oils, are used as feedstock and are esterified to form biodiesel. The simplest approach uses a basic esterification reaction with methanol, sodium hydroxide, and heat. The reaction esterifies the fatty acids of the oil, producing fatty acid methyl esters (FAMEs), which is the biodiesel fuel. Harmful impurities, such as unreacted acylated and free glycerols, must be removed to avoid fuel system damage, for example, fuel filter clogging or fuel injector damage.

The determination of total glycerols (acylated and free glycerols) in biodiesel is challenging. These impurities do not possess chromophores, which precludes the use of ultraviolet or fluorescent HPLC detectors. In addition, the analytes are not volatile and require derivatization for determination by gas chromatography (GC), which is the current industry standard technique.

The ASTM method, D6584, uses N-methyl-N-trimethyl-silyltrifluoroacetamide (MSTFA) to volatilize the glycerols for high-temperature GC. In the synthetic process, methanol and sodium hydroxide are added to the oil, and heat is applied to hydrolyze the oil glycerides and then convert the resulting free fatty acids to FAMEs. After the reaction is complete, the remaining unreacted glycerides, glycerol, methanol, and sodium are washed from the product with water. The reaction yield/quality cannot be determined until the fuel is dried. The derivatization reaction requires a dry sample that cannot contain any residual methanol from the synthetic process, otherwise the derivatization reaction will be impaired or quenched. The reaction itself is intentionally quenched with hexane, which means that this GC determination also cannot be used with petroleum-mixed biofuels. These limitations restrict the GC method to only finished B100 fuel testing, leaving research and development, method optimization, and mixed fuels without an analytical tool.

High pressure liquid chromatography (HPLC) is another means of making these determinations. Evaporative light scattering, mass spectrometry, and pulsed amperometric detectors are possible technologies for use with this analysis, but each has its own limitations and requirements that restrict their use. A typical LC process for these determinations includes the extraction of the free glycerol for analysis, and then saponification of the acylated glycerols for a second analysis by IC-PAD[1] or HPLC-IC-PAD.[2] This requires lengthy sample preparation, and the accuracy of the results will be affected through the process.

The simple, normal-phase HPLC with charged aerosol detection method described here, based on previous work conducted at the USDA,[3] provides a measurement of all acylated and free glycerols. Any biodiesel sample, in-process, finished or blended, is diluted and analyzed directly in under 25 minutes. The method also provides the necessary sensitivity to quantify total glycerols to the current ASTM specifications.

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The sensitivity is possible with the use of the Thermo Scientific Dionex Corona ultra RS charged aerosol detector, which is a universal, mass-based detector. The detector is routinely used for any HPLC analysis and excels where analytes are non-volatile and lacking a chromophore. The functioning of the detector is described in Figure 1.

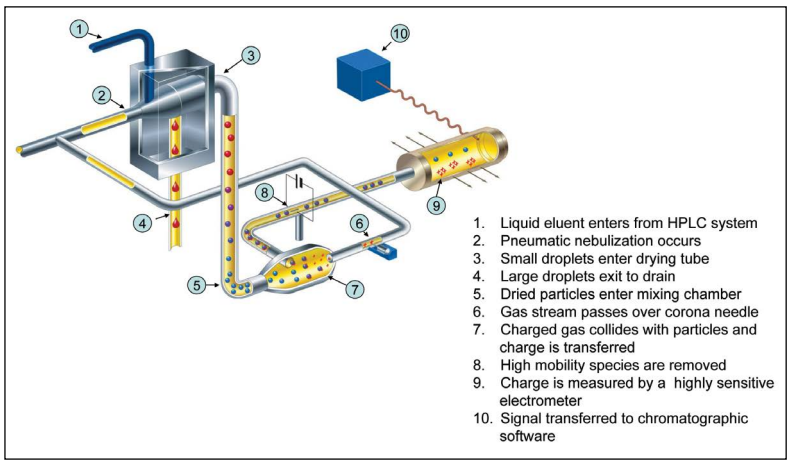

Figure 1. Schematic and functioning of charged aerosol detection

Experimental

A single, normal phase HPLC method, using a cyanopropyl column and a Corona™ ultra RS™ charged aerosol detector, was developed with sensitivity exceeding ASTM requirements. Samples require only dilution prior to analysis.

Sample Preparation

Samples and standards were dissolved in 5 v/v-% isopropanol in 2,2,4-trimethylpentane (TMP). Up to 10% isopropanol in TMP can be used with a 5 µL injection volume.

Standard stock solutions of acylglycerols were prepared at a concentration of 10 mg/mL in TMP and glycerol at 40 mg/mL in isopropanol. Calibration solutions were prepared at a concentration of 250 µg/mL in TMP/isopropanol (95:5) and diluted sequentially.

Liquid Chromatography	
HPLC System:	Thermo Scientific Dionex UltiMate LPG-3400SD HPLC, normal phase
HPLC Column:	Cyanopropyl, 3 µm, 4.0 x 150 mm, ES Industries P/N 234121-EXN-TF
Column Temperature:	40 °C
Mobile Phase A:	2,2,4-trimethylpentane
Mobile Phase B:	Methyl-tert-butyl ether/acetic acid (1000:4)
Mobile Phase C:	2,2,4-trimethylpentane/n-butyl acetate/methanol/acetic acid (500:167:333:4)
Flow Rate:	1.0–1.2 mL/min
Detector:	Corona ultra RS charged aerosol detector
	Power Function (0–12.5 minutes): 2.0
	Power Function (12.5–24.0 minutes): 1.4
	Nebulizer Temperature: 15 °C
	Filter Setting: 5
Sample Temperature:	15 °C
Injection Volume:	2–10 µL
Gradient:	Table 1

Table 1. LC gradient

Time (min)	Flow Rate (mL/min)	%A	%B	%C	Analytes
0.0	1.0	100	0	0	Diesel
2.0	1.0	97	3	0	FAMEs
5.0	1.0	93	7	0	Triacylglycerols
8.0	1.0	60	40	0	Diacylglycerols
9.0	1.0	35	65	0	Monoacylglycerols
10.0	1.0	10	90	0	
10.1	1.0	75	0	25	
11.0	1.2	40	0	60	Glycerol
13.5	1.2	30	0	70	
13.7	1.2	30	70	0	Wash off
15.0	1.2	40	60	0	
16.0	1.2	100	0	0	Re-condition
22.0	1.0	100	0	0	

Data Analysis

All HPLC chromatograms were obtained and compiled using Thermo Scientific Dionex Chromeleon software version 6.8.

Results and Discussion

Calibration

An HPLC chromatogram at 5,000 ng on column (o.c.), is shown in Figure 2. The new "Power Function" parameter of the Corona ultra RS charged aerosol detector was used to provide linear calibration curves, which also improves analytical quantitation, especially for unresolved, compounded peaks. A Power Function value of 2.0 was used for the acylated glycerols, and a value of 1.4 was used for the free glycerol.

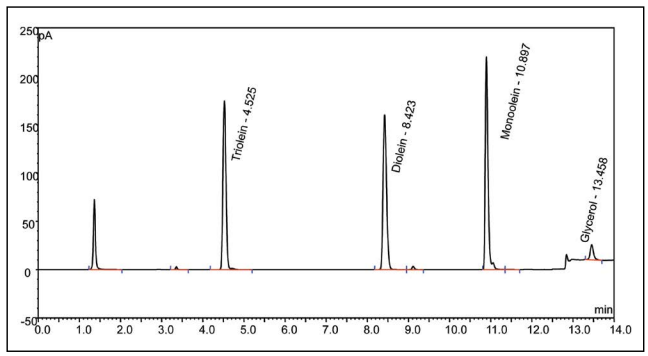

Figure 2. HPLC chromatogram of triolein, 1,3-diolein, monoolein, and glycerol in TMP/isopropanol (95:5) at 5,000 ng o.c.

Each solution was analyzed in triplicate, and the results were plotted with linear regression lines, as shown in Figure 3. All fits were of high correlation, with coefficients r^2>0.999 for all analytes from 39–2,500 ng on column. Replicate injection area-percent RSD values were 0.2–1.5 for the acylglycerols and 2.1–9.7 for the free glycerol for all amounts >78 ng o.c. The calibration precision and method sensitivity results are presented in Table 2. The limits of quantitation (LOQ) were ≤0.0003 mass-percent for the acylglycerols and 0.005 mass-percent for free glycerol, exceeding the requirements specified in ASTM D6584.

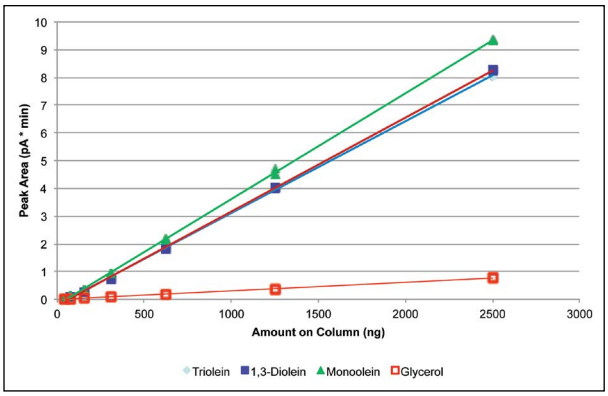

Figure 3. Linear calibration curves for acylated glycerols and free glycerol, 39–5,000 ng o.c

Table 2. Calibration precision and sensitivity values, limit of detection (LOD) and LOQ for the acylated and free glycerols, with mass-percents based on 880 µg biodiesel injection

Analyte	%RSD	LOD		LOQ	
		(ng o.c.)	mass-%	(ng o.c.)	mass-%
Triolein	3.16	1.0	0.0001	3.3	0.0003
1,3-Diolein	3.38	<1.0	<0.0001	1.0	0.0001
Monoolein	2.51	<1.0	<0.0001	1.0	0.0001
Glycerol	3.74	15	0.002	40	0.005

Sample Analysis

The method is capable of quantifying acylglycerols and free glycerol in all biodiesel samples, including in-process, finished B100, and mixed petroleum biodiesels (B5, B10, B20, etc.). Unlike the HT-GC method, which is limited to only finished B100 due to the quenching of the derivatization reagent by methanol or alkanes, the HPLC method only requires that the sample be diluted prior to analysis.

A sample of B20 was prepared with in-process biodiesel by adding 20 µL (17.6 mg) of in-process biodiesel to 80 µL of petroleum diesel and 900 µL of TMP/isopropanol (90:10). An injection volume of 5 µL was used. The chromatogram is shown in Figure 4. Samples containing a greater abundance of glycerols required a greater percentage of isopropanol in the sample solvent. Interestingly, the triacylglycerols showed less retention when more than 0.5 µL of isopropanol was injected. The elution order for the different classes of analytes were: petroleum diesel, the FAMEs of biodiesel, triacylglycerols, 1,3- and 1,2-diacylglycerols, monoacylglycerols, and free glycerol.

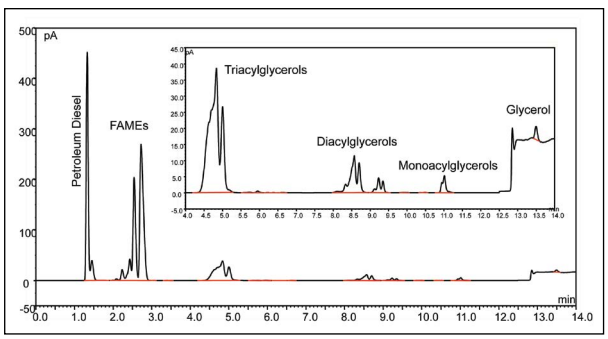

Figure 4. Chromatogram of B20 biodiesel, prepared with in-process biodiesel. Glycerols expanded in inset.

Compared to the previous method,[4] this method provides for greater sensitivity with a significantly shorter analysis time. The use of the linear calibration curves also provides for the direct and straightforward calculation of compound peak impurity results, as demonstrated through the spike recovery calculations below.

To confirm method accuracy, B20 samples prepared with in-process biodiesel were spiked with 500 ng of each glycerol standard and analyzed. Overlaid chromatograms of the spiked and unspiked samples are shown in Figure 5. Recovery values were between 92.8 and 106.9%, as shown in Table 3. This is a marked improvement over our previous method where the recoveries for B100 were between 89.1% and 107%. Furthermore, the current method uses only three eluents and the analysis time was reduced from 40 minutes to 24 minutes, while improving the quantitation limits.

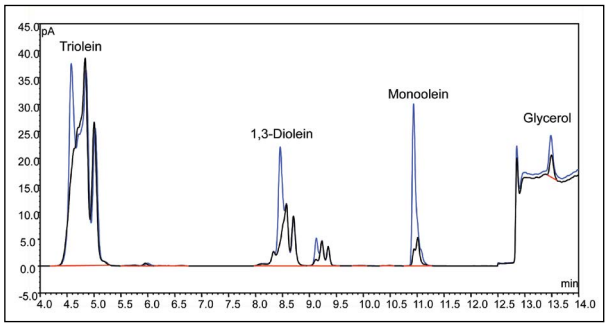

Figure 5. Overlaid chromatograms of B20 biodiesel (black), spiked with 500 ng o.c. of glycerols standards (blue)

Table 3. Percent recovery values using B20 (in-process biodiesel) samples (88 µg o.c.), spiked with 500 ng o.c. (0.56 mass-%) glycerols standard solution

Analyte	Sample (ng)	Spiked Sample (ng)	Recovery (%)
Triolein	3877 (4.4%)	4314 (4.9%)	98.6
1,3-Diolein	846 (1.0%)	1249 (1.4%)	92.8
Monoolein	202 (0.2%)	724 (0.8%)	103.1
Glycerol	1073 (1.2%)	1681 (1.9%)	106.9

Compared to the relatively complex GC chromatogram (not shown), the HPLC chromatogram is very simple: all of the analytes of a similar class (i.e. triacylglycerols) are grouped together. The acylated glycerol peaks in GC tend to be scattered and mixed throughout the chromatogram, making peak assignments difficult.

Conclusion

A single, normal phase HPLC method, using a cyanopropyl column and a Corona ultra RS charged aerosol detector, was developed with sensitivity exceeding ASTM requirements. Samples require only dilution prior to analysis. This improves the accuracy of the method, eliminating the need for internal standards.

The method describes a straightforward means of determining both free and acylated glycerols with only sample dilution and analysis. Samples can be of any form of biodiesel, including in-process, finished biodiesel, as well as petroleum-mixed. The HPLC equipment itself was unspecialized and operated under typical, normal-phase conditions.

- The method provided a total glycerols analysis for all biodiesel samples, which no other method is capable of doing, with examples of in-process, petroleum mixed B20 shown.
- The use of traditional HPLC conditions, combined with simple sample dilution, improved the robustness of this method compared to current GC techniques.
- Because there is no sample extraction or derivatization, no internal standards are required.
- Chromatography is simple, with all analytes of a similar class grouped together.
- The sensitivity of the method exceeds the requirements specified in ASTM D6584.
- Quantitation is greatly improved for the grouped analytes due to linear calibration curves provided by the use of the Power Function available in the Corona ultra RS detector.

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