

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

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## Overview

**Purpose:** To develop an HPLC method to determine acylated and free glycerols in biodiesel (including in process, finished product, and petroleum mixed samples).

**Methods:** A single, normal phase HPLC method, using a glass-lined cyanopropyl column and a Thermo Scientific Dionex Corona charged aerosol detector, was developed with sensitivity exceeding ASTM requirements. Samples require only dilution prior to analysis.

**Results:** An improved shorter and simpler method was created to replace the current method, and results for a B20 sample using this new method are shown.

## Introduction

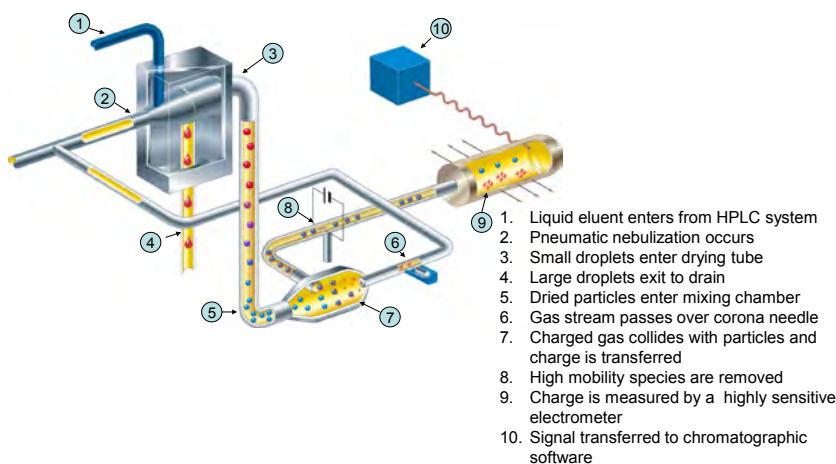
Biodiesel provides a clean and renewable liquid fuel that can be used in current diesel engines and oil burners without any or significant modification to either of them. Natural oils (e.g., virgin and waste cooking oils, algal oils) are used as feedstock and are esterified to form biodiesel. The simplest 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 (fuel filter clogging, fuel injector damage, etc.).

The determination of total glycerols (acylated and free glycerols) in biodiesel is challenging: these impurities do not possess chromophores precluding the use of ultraviolet or fluorescent HPLC detectors, and the analytes are also 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-trimethylsilyltrifluoroacetamide (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 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 which prevent 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<sup>1</sup> or HPLC-IC-PAD.<sup>2</sup> This requires lengthy sample preparation, and the accuracy of the results will be affected through the process.

**FIGURE 1. Schematic and functioning of charged aerosol detection.**



The simple, normal-phase HPLC with charged aerosol detection method described here, based on previous work conducted at the USDA,<sup>3</sup> 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,.

The sensitivity is possible with the use of the Corona ultra RS 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.

## Methods

### Sample Preparation

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

### Liquid Chromatography

HPLC System: Thermo Scientific Dionex Ultimate LPG-3000 HPLC, normal phase  
HPLC Column: ES Industries GLT HPLC column Exsil™ CN, 3  $\mu$ m, 4.6 x 150 mm, titanium frits.  
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: Thermo Scientific Dionex Corona ultra RS  
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  $\mu$ L

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	90	10	0	Triacylglycerols
10.0	1.0	40	60	0	Diacylglycerols
11.0	1.0	40	60	0	Monoacylglycerols
11.0	1.0	100	0	0	
11.2	1.0	60	0	40	
12.0	1.2	35	0	65	Glycerol
15.0	1.2	25	0	75	
16.0	1.2	0	0	100	Wash off
17.0	1.2	0	0	100	
18.0	1.2	0	100	0	
19.0	1.2	100	0	0	Re-condition
24.0	1.0	100	0	0	

### Data Analysis

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

## Results

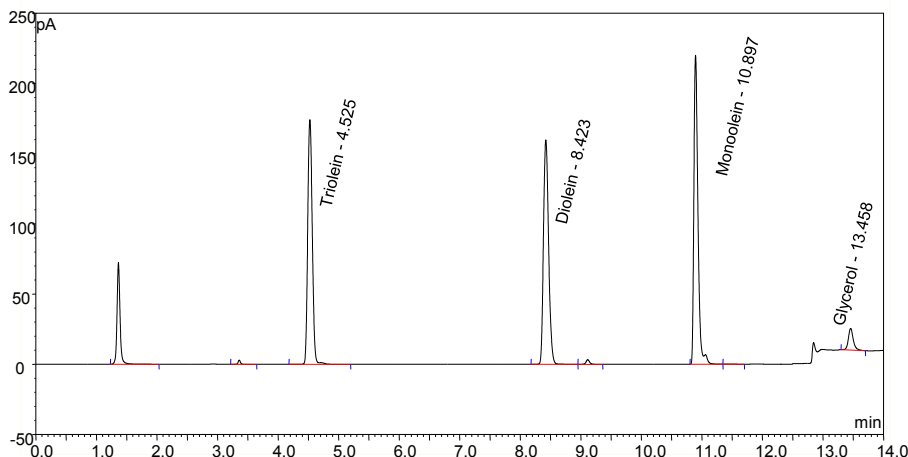
### Calibration

Standard stock solutions of acylglycerols were prepared at a concentration of 10.0 mg/mL in TMP and glycerol at 40 mg/mL in isopropanol. Calibration solutions were prepared at a concentration of 250  $\mu$ g/mL in TMP/isopropanol (95:5), and diluted sequentially. An HPLC chromatogram at 500 ng o.c., is shown in Figure 2.

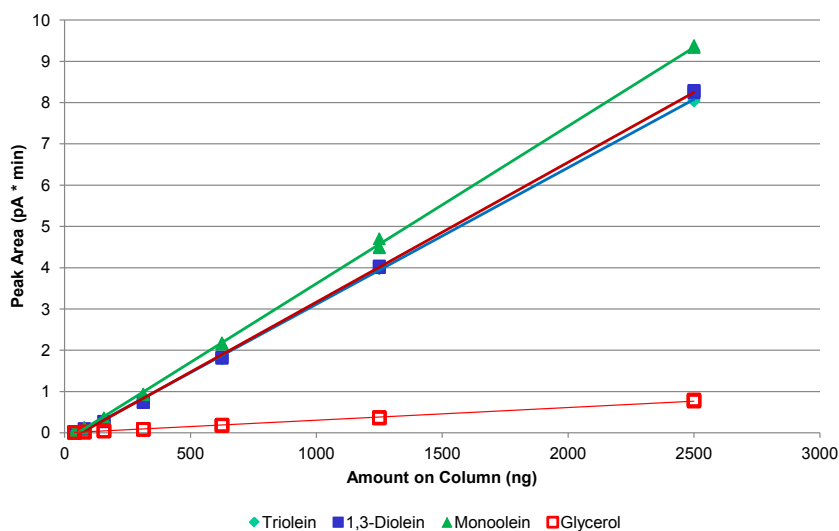
The new “Power Function” parameter of the Corona ultra RS charged aerosol detector was used to provide linear calibration curves, which also improves analytical quantitation, especially for unresolved compound peaks. A Power Function value of 2.0 was used for the acylated glycerols, and a value of 1.4 was used for the free glycerol.

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 – 2500 ng o.c. 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 1. The limits of quantitation (LOQ) were  $\leq 0.0003$  mass-percent for the acylglycerols and 0.005 mass-percent for free glycerol, exceeding the requirements specified in ASTM D6584.

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



**FIGURE 3. Linear calibration curves for acylated glycerols and free glycerol, 39–5000 ng o.c.**



**Table 1. Calibration precision and sensitivity values, limit of detection (LOD) and LOQ for the acylated and free glycerols, with mass-percents based on 880  $\mu\text{g}$  biodiesel injection.**

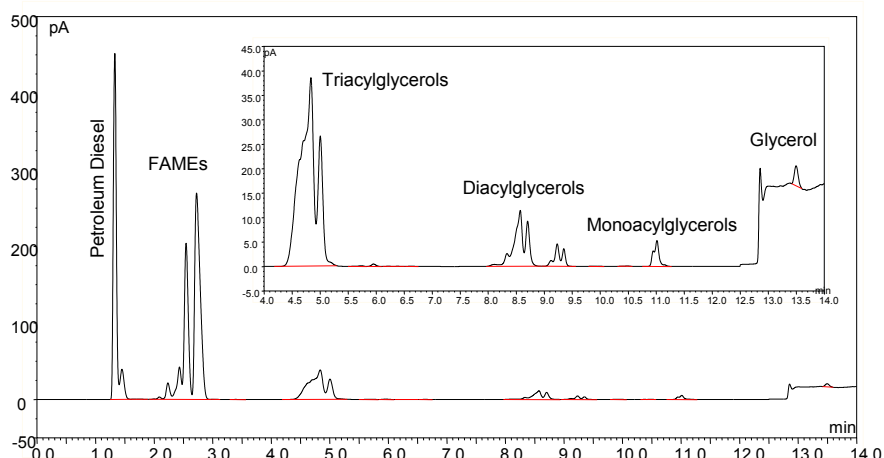
Analyte	%RSD	LOD		LOQ	
		(ng o.c.)	mass-%	(ng o.c.)	mass-%
Triolein	3.16	1.0	0.0001	3.3	0.0003
1,3-Diolein	3.38	< 1	< 0.0001	1.0	0.0001
Monoolein	2.51	< 1	< 0.0001	1.0	0.0001
Glycerol	3.74	15	0.002	40	0.005

### 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 only to 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  $\mu\text{L}$  (17.6 mg) of in-process biodiesel to 80  $\mu\text{L}$  of petroleum diesel and 900  $\mu\text{L}$  of TMP/isopropanol (90:10). An injection volume of 5  $\mu\text{L}$  was used, and 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  $\mu\text{L}$  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,<sup>4</sup> this method provides for greater sensitivity with a significantly shorter analysis time, and requires only three mobile phases. 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 2. This is a marked improvement over our previous method where the recoveries for B100 were between 89.1 – 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.

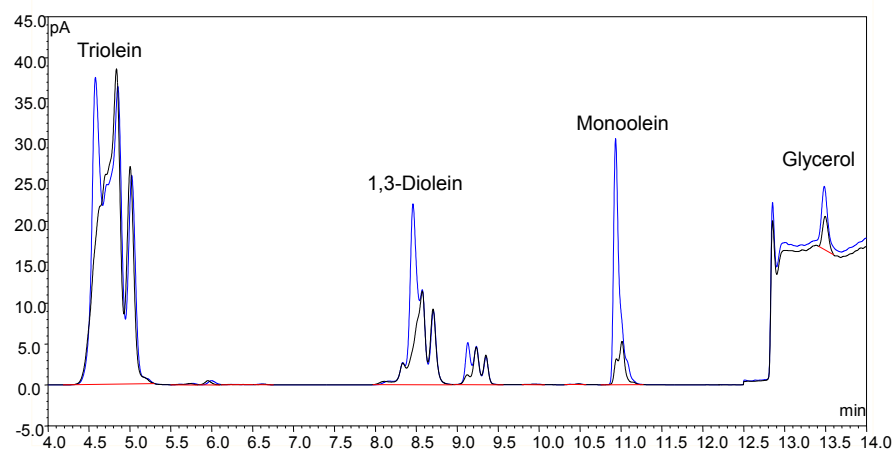
Compared to the relatively complex GC chromatogram (not shown), the HPLC chromatogram is very simple: all of the analytes of a similar class (i.e. triacylglycerols) are grouped together. The acylated glycerol peaks in GC tend to be scattered and mixed throughout the chromatogram, making peak assignments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

Sample preparation is straightforward, with only a dilution of the sample. This improves the accuracy of the method, eliminating the need for internal standards. Unlike the GC method, which requires a cool-on-column injector, high temperature GC columns, and usually a derivatization-capable autosampler, the HPLC instrument is unspecialized, and operates under typical conditions.

**Table 2. Percent recovery values using B20 (in-process biodiesel) samples (88  $\mu\text{g}$  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

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



## Conclusion

The method is an update to previous work, and uses a shorter column with a smaller, 3  $\mu\text{m}$  particle diameter packing material, fewer eluents, and showed an increased scope in samples, capable of quantifying total glycerols in all biodiesel samples.

- 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, improves 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 the use of the Power Function available in the Corona ultra RS detector.

## References

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4. Application Note 70-8305, "Total Glycerides of Biodiesel by Normal Phase HPLC and Corona ultra", 70-8305P Rev C, 2008, Dionex, Part of Thermo Fisher Scientific, <http://www.coronaultra.com/Applications/Notes/70-8305-Total-glycerols-of-Biodiesel-by-Normal-Phase-HPLC-and-corona-ultra.pdf> (accessed Feb. 24, 2012).

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