

# Efficient Quantitation of Ion and Total Glycerin Impurities in Biodiesel Using HPLC-CAD

Bruce Bailey, Marc Plante, Christopher Crafts, Paul Gamache, John Waraska, and Ian Acworth  
ESA - A Dionex Company, Chelmsford, MA USA



## ABSTRACT

Ionic impurities in biodiesel, such as sodium, potassium, calcium, and magnesium, form abrasive particles that can cause excessive wear in moving parts. They also form soaps which can lead to filter blockage and engine deposits. The standardized test for these ions, EN14538, uses expensive inductively coupled plasma for optical emission spectral analysis (ICP-OES) instrument. The established limits of combined sodium and potassium, and combined calcium and magnesium concentrations are no more than 5 ppm.<sup>1</sup> The authors have developed a method using HPLC and Corona<sup>®</sup> *ultra*<sup>™</sup> Charged Aerosol Detector (CAD<sup>®</sup>) with zwitterionic column technology and a binary gradient. Impurity-spiked samples of B100 were prepared and analyzed to quantify each of these ions down to 1 ppm concentration in less than 25 min. Free glycerin is also quantifiable down to the 0.02 % w/w requirement.

A second method using a similar HPLC system was developed for the determination of total glycerins, including mono-, di-, triacylglycerins, and free glycerol in one HPLC-CAD analysis, without derivatization, or the need for expensive columns and specialized equipment. Biodiesel samples were diluted in an isooctane/isopropyl alcohol solution before direct injection. All detection limits satisfied and exceeded requirements needed for ASTM D6584 approval: LOQ values of 0.002 w/w-% for acylated glycerins and 0.006 w/w-% for free glycerol were determined for this method. Sample results obtained from this HPLC method are comparable to results obtained from GC analysis.

## INTRODUCTION

During the production of biodiesel from the base-catalyzed transesterification of vegetable and animal fats, the contaminants glycerol and acylated glycerins are produced. These contaminants have been found to foul fuel filters and ignition systems at relatively low concentrations. The current analytical technique to determine these contaminants uses high temperature gas chromatography (HT-GC), which requires sample derivatization, a specially equipped GC instrument, and high temperature columns (with limited lifetimes in practical use). This requires specialized instrumentation, reagents, and analyst time. HPLC has been cited as being a lower cost alternative.<sup>2</sup> Ions, mainly from the basic catalyst but also from feedstock and water, can remain as contaminants in the product. A second method has been developed that can separate and show the presence of different ions in biodiesel including sodium, potassium, magnesium, calcium, and sulfate.

The authors have developed simple methods capable of measuring glycerin impurities and ions in biodiesel using one analytical platform. These methods use a normal phase HPLC system for the determination of total glycerins (based on prior work performed at the USDA<sup>3</sup>), and a zwitterionic hydrophilic interaction (HILIC) system for the detection of free glycerin, cations, and anions, using the Corona *ultra* CAD. Both of these systems can be run on a single HPLC system, and the Corona *ultra* detector exhibits the necessary sensitivity for these analytes to meet the quantitative requirements of standard methods (ASTM and EN).

The use of the Corona *ultra* detector, a highly sensitive, mass-based detector, offers flexibility of analysis, and the performance specifications to meet challenging analyses.

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## EXPERIMENTAL

### Total Glycerins by NP-HPLC-CAD

#### Corona ultra Parameters:

Gas: 35 psi via nitrogen generator  
Filter: Corona  
Range: 500 pA  
Nebulizer Heater: 30 °C

#### HPLC Parameters:

Mobile Phase A: Iso-octane/acetic acid (1000:4)  
Mobile Phase B: Iso-octane/isopropyl alcohol/acetic acid (1000:1:4)  
Mobile Phase C: Methyl-*t*-butyl ether/acetic acid (1000:4)  
Mobile Phase D: Iso-octane/*n*-butyl acetate/methanol/acetic acid (500:666:133:4)

Gradient:

Time	%A	%B	%C	%D	Flow Rate
0.00	100.0	0.0	0.0	0.0	1.00
5.00	0.0	98.0	2.0	0.0	1.00
7.00	0.0	95.0	5.0	0.0	1.00
15.00	0.0	92.0	8.0	0.0	1.00
17.00	0.0	65.0	35.0	0.0	1.00
23.00	0.0	50.0	50.0	0.0	1.00
23.10	80.0	0.0	0.0	20.0	1.00
25.00	10.0	0.0	0.0	90.0	1.00
28.00	10.0	0.0	0.0	90.0	1.00
28.10	20.0	0.0	80.0	0.0	1.00
29.00	20.0	0.0	80.0	0.0	1.00
29.50	100.0	0.0	0.0	0.0	1.20
39.00	100.0	0.0	0.0	0.0	1.20
39.50	100.0	0.0	0.0	0.0	1.00

Flow Rate: 1.0–1.2 mL/min  
Run Time: 40 min  
HPLC Column: SGE Exsil CN, 250 × 4.0 mm; 5 µm  
Column Temperature: 30 °C  
Sample Temperature: 10 °C  
Injection Volume: 10 µL  
Sample Preparation: B100 biodiesel samples were prepared by dissolving 100 µL of biodiesel in 900 µL of iso-octane/isopropyl alcohol (98:2) and mixed in an HPLC vial.

### Glycerin and Ions by HPLC-CAD

#### Corona ultra Parameters:

Gas: 35 psi via nitrogen generator  
Filter: None  
Range: 100 pA  
Nebulizer Heater: Off

#### Analytical Conditions:

Column: SeQuant ZIC®-pHILIC, 150 × 4.6 mm, 5 µm  
Mobile Phase A: 100 mM ammonium formate pH 2.9/acetonitrile/isopropyl alcohol/ethanol (15:60:25:5)  
Mobile Phase B: 200 mM ammonium formate pH 2.9/acetonitrile/isopropyl alcohol/methanol (30:40:25:5)  
Flow Rate: 0.65 mL/min  
Injection Volume: 50 µL  
Run Time: 30 min

Gradient:

Time (min)	% B
0	20
2	20
20	70
25	70
26	20

#### Extraction Method

Add 25 mL of a B100 biodiesel sample and 25 mL of hexane to a 125 mL separatory funnel. Add 5 mL extraction solution (1:1 methanol/0.5% formic acid in water) and mix. After the solution settles, remove the bottom layer and repeat wash as needed. The data presented in Figure 4 used four extractions.

#### Filtration and Sample Preparation

Add a 500 µL aliquot of aqueous extract to centrifuge tube filter (0.22 µm) and centrifuge. Add a 200 µL aliquot extract to 800 µL of acetonitrile, mix, and then inject.

# RESULTS

## Calibration Curves

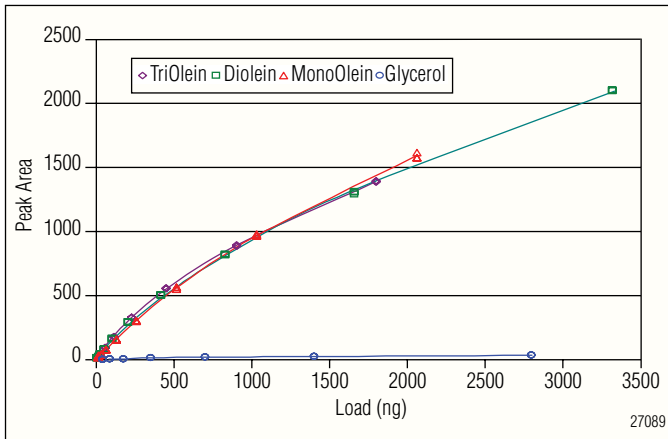


Figure 1. Calibration curves for free and acylated glycerols from 6 to 3320 ng on the column. All RSD values < 5%.

## Impurity Retention Time Ranges

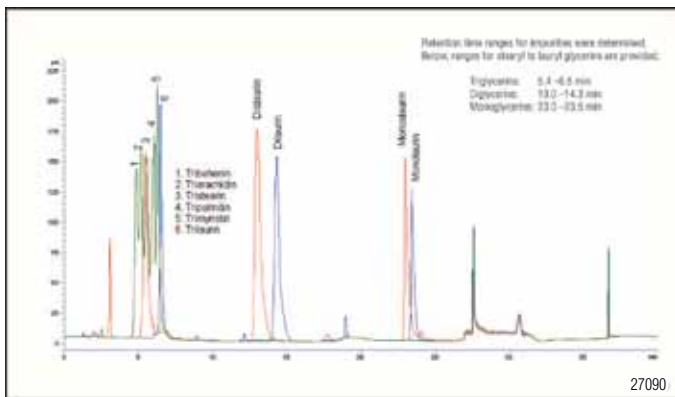


Figure 2. Chromatograms of acylated glycerin standards, including variations in carbon chain length.

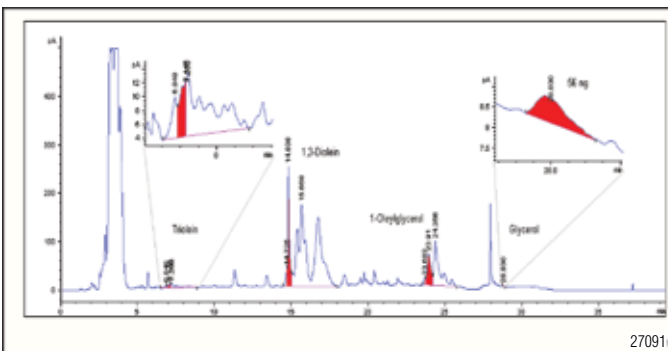


Figure 3. 880 µg on column of B100 biodiesel sample.

Table 1: Total Glycerins Samples Analysis: HPLC Method vs. GC ASTM Method				
Impurity	HPLC Found (µg)	HPLC Found (w/w %)	Adjusted <sup>1</sup> HPLC Found (5)	GC <sup>2</sup> ASTM Found (%)
Triolein	0.0645	0.00733	0.0008	< 0.002
1,3 -DiOlein	2.617	0.2974	0.0440	0.0510
1 -Oleylglycerol	1.117	0.1269	0.0367	0.0300
Glycerol	0.061	0.0069	0.0069	< 0.002
<b>Total</b>			<b>0.088</b>	<b>0.081</b>

<sup>1</sup>Values adjusted using ASTM factors from ASTM D6584.

<sup>2</sup>Values obtained from same sample using ASTM D6584.

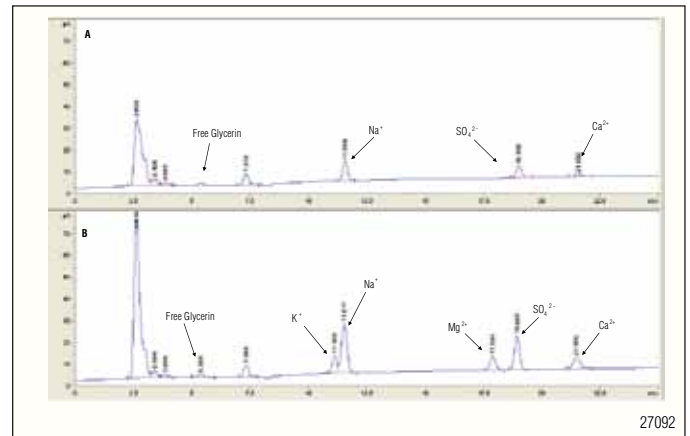


Figure 4. A) Chromatogram of an aqueous extraction of biodiesel or B100. (Found: free glycerin 19, sodium 1.1, and sulfate 2.4 ppm). B) Chromatogram of the sample solution spiked with free glycerin 400, potassium 3.8, sodium 6.8, magnesium 4.0, sulfate 16, and calcium 6.2 ppm.

## DISCUSSION AND CONCLUSIONS

The results for the total glycerins in the 100% biodiesel sample, and a comparison of an HT-GC ASTM D6584 determination of the same biodiesel are provided in Table 1. Sample preparation for the total glycerins only requires a simple dilution in iso-octane/isopropyl alcohol (98:2), avoiding derivatization and the need for internal standards.

The HPLC-CAD method for total glycerins shown here is capable of providing both accurate and precise data for all the glycerin impurities, bound and free, in biodiesel using a single run. Compared to the current ASTM method, sample preparation is simplified, and uses typical unspecialized HPLC equipment that can be used for other analytical methods, such as for the analysis of ions in biodiesel. The Ions method allows for the determination of ions in a biodiesel sample, and both methods use mobile phase systems that are compatible with mass spectrometry to identify other peaks that may be of concern.

Calibration curves for acylated and free glycerins are shown in Figure 1. The acylated glycerins have similar calibration curves. Different carbon chain-lengths within a class of acyl-glycerols have only a moderate effect on retention time by NP-HPLC, as shown in Figure 2. This helps to simplify peak identification, contrasting the complex GC chromatograms. An analysis of a B100 sample, presented in Figure 3, shows the presence of glycerin contaminants.

For sensitivity with free and acylated glycerins, the LOQ values for all of the acylated glycerins are 15 ng on column (0.002 w/w-%) and for the glycerol 50 ng on column (0.006 w/w-%), satisfying ASTM requirements. Ionic contaminants can also be detected using HPLC-CAD, using the second method. A spiked sample of B100 showed the presence of the spiked ions and increases in peak area for those already present in the unspiked sample, as shown in Figures 4A and 4B. As the sample preparation conditions are not finalized, only qualitative data is provided. The sensitivity of the HPLC method using CAD provides the basis to meet the requirements outlined by EN14214 for ions.

The NP-HPLC method produced results that are comparable to results obtained from the HT-GC method, with total glycerins by HPLC determined at 0.088%, compared to 0.081% by HT-GC.

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### Dionex Corporation

1228 Titan Way  
P.O. Box 3603  
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94088-3603  
(408) 737-0700

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