

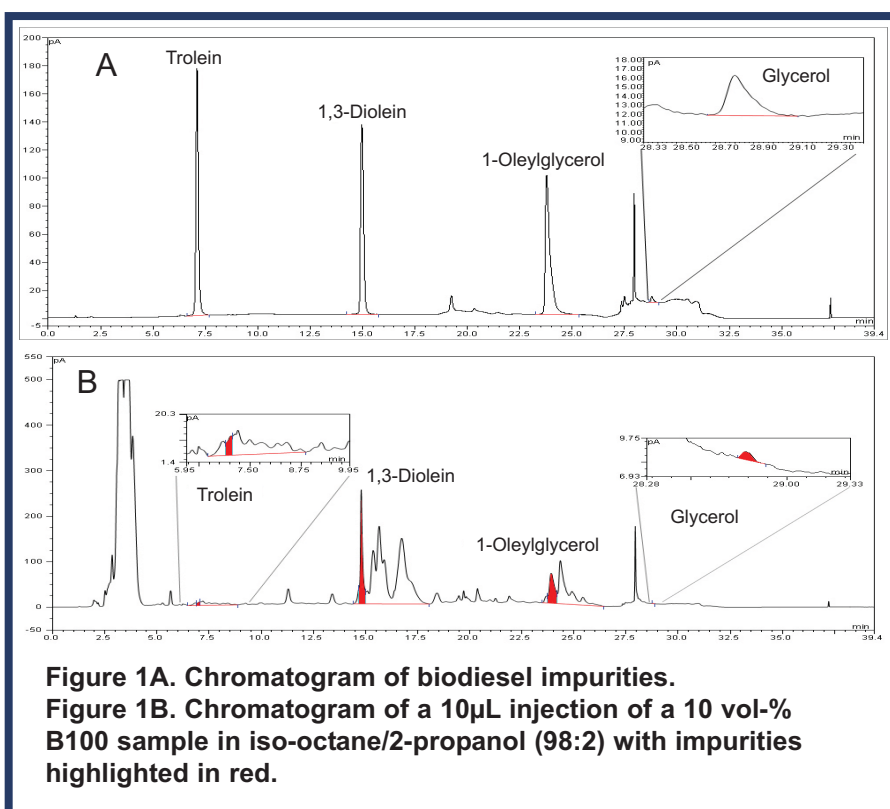
# Total Glycerides of Biodiesel by Normal Phase HPLC and Corona *ultra*

Biodiesel is a renewable fuel produced from the esterification of vegetable and animal fats. During this base-catalyzed process, free glycerol and mono-, di-, and triacylglycerols are produced which are contaminants harmful to combustion systems. As a result, these impurities are limited to low levels (0.02% glycerol, 0.240% total glycerides) as determined by the current high-temperature gas chromatography method, ASTM D6584. This application note describes the use of charged aerosol detection in Normal-Phase HPLC for the determination of glycerol, and mono-, di-, and tri-acyl-glycerol impurities in biodiesel without requiring sample derivatization, internal standards, and additional or specialized equipment. The Corona *ultra* is a sensitive, mass-based detector ideally suited to meet these analytical requirements.

This HPLC-based method:

- Requires only a “common” HPLC system
- Provides accurate and precise results with external standardization
- Does not require sample derivatization
- Does not require a special chromatography column;
- Does not require the use of internal standards
- Has low ng LODs for all analytes

This method, based on the work of Foglia and Jones (J. Liq. Chrom. Rel. Tech., **20**, 1829-1838 1997), can provide accurate and precise determinations of these impurities. Chromatographic parameters for sample analysis are provided on the next page.



Impurity standards, containing glycerol and the three acylated glycerols in 2,2,4-trimethylpentane (iso-octane)/2-propanol (98:2) were used to standardize detector response. A representative chromatogram is shown in Figure 1A. Sample preparation is greatly simplified: dilution in iso-octane/2-propanol (98:2), without the need for internal standards or derivatization. Using this HPLC-CAD method, the preparation times are greatly reduced compared to those of the ASTM GC method. Shown in Figure 1B is a chromatogram of a biodiesel sample at 10-vol% B100\*. Simplified sample manipulation (dilute and shoot), helps save time and lowers sample preparation costs.

This method was used with a typical column load for biodiesel of 880 $\mu$ g. This allows for quantization of the four analytes with LOQ and LOD values shown in Table 1. Greater loads will lower these values further but these are sufficient for testing for the ASTM requirements. LOQ was determined at a column load that yielded <15% RSD, and LOD was taken at approximately 1/3 of the LOQ value.

Analyte	LOQ (ng)	LOQ (w/w-%)	LOD (ng)	LOD (w/w-%)
Triolein	15	0.002	5	0.0006
1,3-Diolein	15	0.002	5	0.0006
1-Oleylglycerol	15	0.002	5	0.0007
Glycerol	50	0.006	20	0.002

**Table 1. LOQ and LOD values for different analytes.**

## Method Parameters

Column: \*SGE Exsil CN 250 x 4.0 mm, 5 µm, 35°C  
 Nebulizer Heater: 30°C  
 Filter: None  
 Mobile Phase A: iso-Octane/HOAc (1000:4)  
 Mobile Phase B: iso-Octane/2-Propanol/HOAc (1000:1:4)  
 Mobile Phase C: Methyl-t-butyl ether/HOAc (1000:4)  
 Mobile Phase D: iso-Octane/n-Butyl acetate/Methanol/HOAc (500:167:333:4)  
 Gradient Profile: Table 2  
 Flow Rate: 1.0-1.2 mL/min  
 Run Time: 40 minutes  
 Injection Volume: 10µL at 10°C  
 Sample Conc: 100µL Biodiesel in 900µL iso-Octane/2-Propanol (98:2)

Time	%A	%B	%C	%D	Flow Rate
0.00	100	0	0	0	1.0
5.00	0	98.0	2.0	0	1.0
7.00	0	95.0	5.0	0	1.0
15.00	0	92.0	8.0	0	1.0
17.00	0	65.0	35.0	0	1.0
23.00	0	50.0	50.0	0	1.0
23.10	80	0	0	20.0	1.0
25.00	10	0	0	90.0	1.0
28.00	10	0	0	90.0	1.0
28.1	20	0	80.0	0	1.0
29.00	20	0	80.0	0	1.0
29.50	100	0	0	0	1.2
39.00	100	0	0	0	1.2
39.50	100	0	0	0	1.0

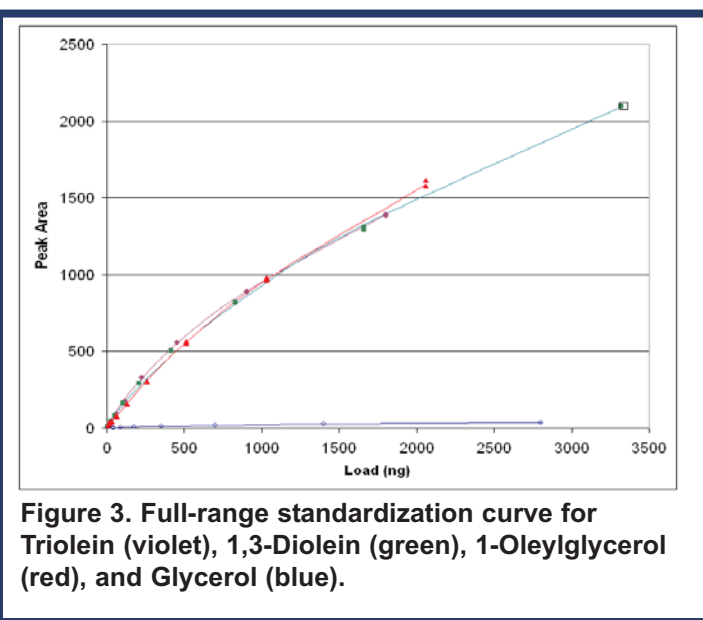
**Table 2. Gradient conditions.**

Due to the complex nature of the sample matrix, a gradient program was created, using four mobile phases: mobile phase A allows for the sample to be loaded without splitting the more polar analytes; mobile phase B provides additional selectivity over mobile phase A with 2-propanol concentrations between 0.1–0.2%; mobile phase C elutes the polar compounds; and mobile phase D elutes the glycerol. Mobile phase C is used again to rinse the column of any remaining D, and then the column is re-conditioned.

## Standardization

Stock standard solutions were prepared in iso-octane, with glycerol dissolved in 2-propanol, at concentrations of 1 mg/mL each. The stock solutions were then diluted together creating a mixed standard solution of approximately 20µg/mL each. The dilution solvent was iso-octane/2-propanol (98:2). Sequential dilutions were made with a dilution factor of 2. Ten microliters of each standard solution was injected in triplicate, yielding the curves shown in Figure 2. Note the uniformity of response across the three acylated glycerol standards.

Curve fitting was performed using an “Inverted Quadratic” fit for the alkylated glycerols and a quadratic fit for glycerol.



**Figure 3. Full-range standardization curve for Triolein (violet), 1,3-Diolein (green), 1-Oleylglycerol (red), and Glycerol (blue).**

# The Corona *ultra* Charged Aerosol Detector

## Sample Analysis

A sample was prepared by dissolving 100µL (88 mg) of biodiesel B100\* in 900µL of iso-octane/2-propanol (98:2). Two injections were made of each sample, and the peak areas were quantified for impurity content, using standardization curves containing at least four standard concentrations containing the sample peak area.

Results for the sample analysis are shown in Table 3. The last two columns of data illustrate that the HPLC method using the ASTM adjustment factors correlates well with the official ASTM GC method.

Impurity	HPLC Found (µg)	HPLC ASTM Found (%)	GC <sup>1</sup> ASTM Found (%)
Triolein	0.0645	0.0008	<0.002
1,3-Diolein	2.617	0.0440	0.0510
1-Oleylglycerol	1.117	0.0367	0.0300
Glycerol	0.058	0.0066	<0.002
<b>Total</b>		<b>0.088</b>	<b>0.081</b>

**Table 3. Sample analysis results, HPLC vs. ASTM GC methods. Values obtained from ASTM D6584.<sup>1</sup>**

## Spike and Recovery

Three spike levels were used, containing the mixed standards at 0.01, 0.02, and 0.05% glycerol. Recovery values were acceptable, between 89 –107% for all four impurities, as shown in Table 4.

Sample		Triolein	1,3-Diolein	1-Oleylglycerol	Glycerol
B100	Found (ng)	64.5	2617	1117	58
B100 + 0.01%	Theoretical (ng)	121	2669	1313	146
	Found (ng)	114	2529	1241	130
	<b>Recovery (%)</b>	<b>94.4</b>	<b>94.8</b>	<b>94.6</b>	<b>89.1</b>
B100 + 0.02%	Theoretical (ng)	178	2721	1377	234
	Found (ng)	169	2715	1281	242
	<b>Recovery (%)</b>	<b>94.7</b>	<b>99.8</b>	<b>93.0</b>	<b>103</b>
B100 + 0.05%	Theoretical (ng)	347	2877	1571	498
	Found (ng)	343	3066	1491	494
	<b>Recovery (%)</b>	<b>98.9</b>	<b>107</b>	<b>94.9</b>	<b>99.2</b>

**Table 4. Recovery values of biodiesel impurities at three concentrations.**

## Conclusions

The developed method has the capacity to determine the glyceride and glycerol impurities both accurately and precisely in biodiesel samples. The HPLC CAD-based method only requires a standard HPLC system with no special chromatography columns. This simpler method does not compromise accuracy, precision or sensitivity. The obtained results are comparable to those obtained by the HT-GC ASTM D6584 method.

## Ordering Information

Corona *ultra*<sup>TM</sup> Charged Aerosol Detector 70-8773  
Nitrogen generator 70-6003

## References

<sup>1</sup> Knothe, G., Analytical Methods Used in the Production and Fuel Quality Assessment of Biodiesel. Transactions of the ASAE. (2001), 44(2), 193-200.

\*ESA wishes to thank SGE, Inc (Austin, TX) for their column and Bursaw Gas & Oil, Inc. (Acton, MA) for providing biodiesel samples.



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