

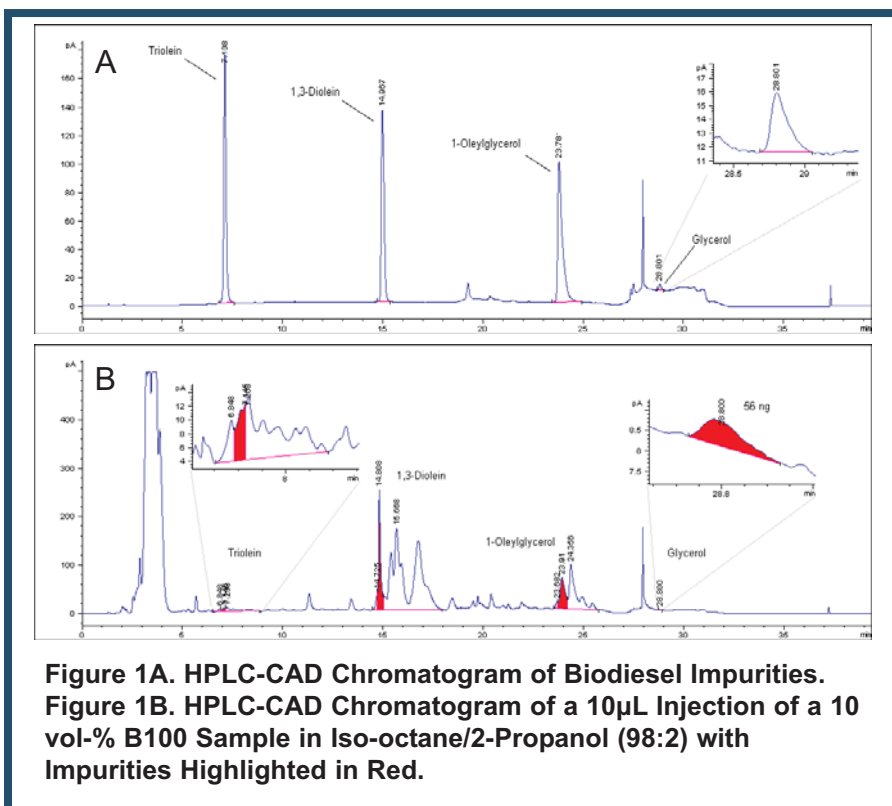
Biodiesel Analysis by Normal Phase HPLC and Corona CAD

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 the Corona charged aerosol detector (CAD) in Normal-Phase HPLC for the determination of glycerol, and mono-, di-, and tri-acylglycerol impurities in biodiesel without requiring sample derivatization, internal standards, and additional or specialized equipment. The Corona CAD is a sensitive, mass-based detector ideally suited to meet these analytical requirements.

This HPLC-based method:

- Only requires 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; and
- Has low ng LODs for all analytes.

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



below. 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, and 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 µg. This allows for quantization of the four analytes with LOQ and LOD values shown in Table 1. Subsequently, greater loads will lower these values further but are sufficient for testing for the ASTM requirements at this load. 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 4.6 mm, 5 µm, 35°C
 Detector: Corona CAD Plus
 Nebulizer Heater: On
 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.

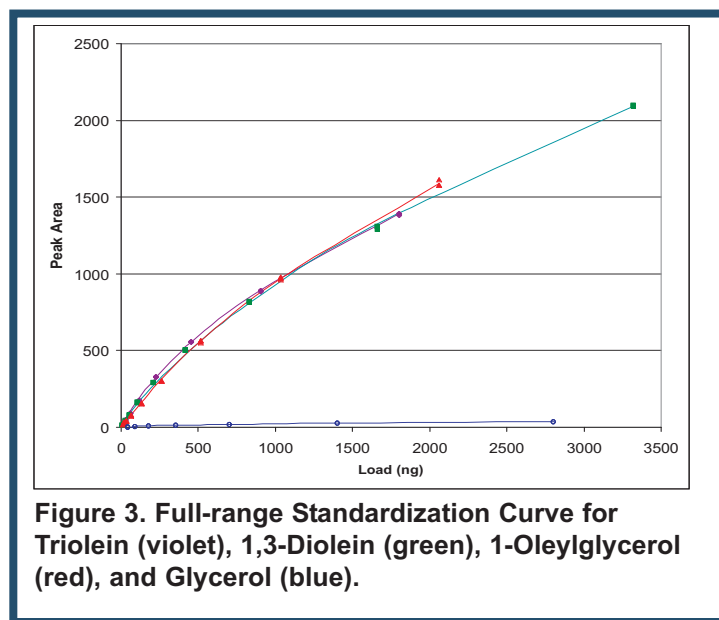


Figure 3. Full-range Standardization Curve for Triolein (violet), 1,3-Diolein (green), 1-Oleylglycerol (red), and Glycerol (blue).

The Corona[®] Charged Aerosol Detector



Sample Analysis

A sample was prepared by dissolving 100 μ L (88 mg) of biodiesel B100* in 900 μ L 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 (μ g)	HPLC ASTM Found (%)	GC ¹ 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
Total		0.088	0.081

Table 3. Sample Analysis Results, HPLC vs. ASTM GC Methods. ¹Values obtained from ASTM D6584.

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
	Recovery (%)	94.4	94.8	94.6	89.1
B100 + 0.02%	Theoretical (ng)	178	2721	1377	234
	Found (ng)	169	2715	1281	242
	Recovery (%)	94.7	99.8	93.0	103
B100 + 0.05%	Theoretical (ng)	347	2877	1571	498
	Found (ng)	343	3066	1491	494
	Recovery (%)	98.9	107	94.9	99.2

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 Corona CAD HPLC-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 Plus Charged Aerosol Detector	70-7041
Thermal Organizer Module	70-5499TA
Nitrogen generator	70-6003
Pump, model 584**	70-7091
Quaternary Low Pressure Gradient	70-5260
Autosampler, model 542	70-4152
EZChrom Elite [™] for ESA software including PC	70-5073

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

**Modification required - contact ESA Technical Support



ESA Biosciences, Inc.
22 Alpha Road
Chelmsford, MA 01824-4171
USA
+1 978.250.7000 Phone
+1 978.250.7090 Fax
+1 800.959.5095 US Toll free
Email: info@esainc.com
www.esainc.com

ESA Analytical
Brook Farm, Dorton, Aylesbury
Buckinghamshire HP18 9NH
England
+44 (0)1844 239381 Phone
+44 (0)1844 239382 Fax
Email: info@esainc.com
www.esainc.com

ESA Biosciences, Inc. is an ISO 9001 and 13485 Certified Company
ESA, Corona and CAD are registered trademarks of ESA Biosciences, Inc.
The Corona CAD is covered by multiple patents

70-8305
Rev A