Determination of Free and Total Glycerol in Biodiesel Samples by HPAE-PAD Chromatography

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Application Note 255

Introduction

Biodiesel fuels are part of a rapidly growing alternative fuel industry using diesel fuel blended with 2–20% (B2–B20) of fatty acid methyl esters (FAMEs) obtained from vegetable or animal oils.[1–5] Although biodiesel is only 0.93–1.5% of the liquid biofuel transportation market dominated by ethanol and ethanol blends, its consumption is growing.[1] From 2000 to 2007, global biodiesel production expanded from <1 billion to 11 billion liters.[1] Biodiesel fuels have the additional benefit of burning more efficiently than gasoline, which results in lower carbon monoxide and carbon dioxide emissions. Based on a U.S. Department of Energy study, replacing gasoline with B20 biodiesel from soy can reduce carbon emissions by 16%.[5] Therefore, some governments have mandated the use of biofuels. Gasoline must contain 20% biofuel by 2022 in the U.S and >6.25% biofuel by 2010 in Germany.[1]

FAMEs are manufactured from various triglyceride feedstocks, including soy, rape seed, and palm oils.[1,2,5] Triglycerides have three aliphatic unbranched fatty acids bonded to one molecule of glycerol, each by an ester linkage. The triglycerides undergo a transesterification reaction, with excess methanol and potassium hydroxide as the catalyst, to generate three molecules of free FAMEs and one molecule of glycerol byproduct.[3,4] The glycerol, excess alcohol, and base are then separated from the FAMEs. The alcohol and base are recycled for another transesterification reaction, whereas the glycerol is refined and sold for use in industrial and medicinal syrup pharmaceutical products. Glycerol is an undesirable byproduct in biodiesel and therefore must be removed from the FAMEs. Free glycerol and bound glycerol from unconverted triglycerides cause negative effects on the diesel engine, such as clogging fuel filters, fouling fuel injectors, and forming a deposit on the bottom of fuel storage tanks.[5] Additionally, the sub-freezing temperatures common in many winter climates affect unconverted triglycerides (bound glycerol) more than that typically observed in diesel. The most common effects are fuel separation and increased fuel viscosity, which results in poor fuel injection and engine lockup.

Total glycerol, defined as free glycerol plus bound glycerol (unconverted triglycerides), is determined by base-hydrolysis to convert the bound glycerol to free glycerol. To minimize the problems caused by free and bound glycerol, sustain production of biofuels, and provide quality control criteria to this developing industry, the ASTM and other regulatory organizations have established limits of 0.02 wt % free glycerol and 0.24 wt % total glycerol (ASTM D6751) in B100 biofuels used to prepare B2–B20 blends.[3,5] Therefore, accurate and sensitive methods are needed for the determination of free and total glycerol in biodiesel.

Glycerol is commonly determined by gas chromatography with flame ionization or mass spectrometry detection. However, it has been reported that glycerol causes the localized degradation of silated columns that are commonly used by this technique. Therefore, samples are derivatized to produce trimethylsilated glycerols to eliminate this problem.[3] High-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) is a well-established method that can determine carbohydrates and glycols without sample derivatization.[3,7,8] In the work presented here, glycerol is determined in biodiesel samples by HPAE-PAD on a 4 × 250 mm Thermo Scientific™ Dionex™ CarboPac™ MA1 column using 100 mM sodium hydroxide eluent and a Au working electrode with a four-potential carbohydrate waveform.

The Dionex CarboPac MA1 column consists of macroporous resin beads with optimized selectivity for the separation of sugar alcohols such as glycerol. This selectivity allows glycerol to be retained longer on this column than other Dionex CarboPac columns, which results in the resolution of glycerol from other compounds. Glycerol elutes from the column within 11 min

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and is fully separated from other early eluting peaks. This method takes advantage of the sensitivity and selectivity of PAD to minimize the amount of sample injected to determine µg/kg to mg/kg concentrations of glycerol in biodiesel samples. Additionally, the method qualification data and sample preparation techniques to determine free and total glycerol in biodiesel samples are presented. The advantages of using HPAE-PAD with the Dionex CarboPac MA1 column to determine free and total glycerol in 10 biodiesel samples are also discussed.

Equipment

- Thermo Scientific™ Dionex™ ICS-3000 ion chromatography system including:
  - DP Dual Pump or SP Single Pump gradient pump module with degas option
  - DC Detector/Chromatography module
  - Thermo Scientific™ Dionex™ AS Autosampler and 1.5 mL sample tray
  - ED Electrochemical Detector (P/N 079830)
  - Electrochemical cell (cell and reference electrode, P/N AAA-061756)
  - Combination pH–Ag/AgCl reference electrode (P/N 061879)
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System software
- Vial Kit, 1.5 mL glass sample vials, with caps and slit septa (vial kit, P/N 055427)
- Vial Kit, 1.5 mL polypropylene sample vials, with caps and slit septa (vial kit, P/N 079812)
- Filter unit for vacuum filtration, 0.20 µm nylon (Nalgene® Media-Plus with 90 mm filter, Nalge Nunc International, P/N 164-0020) or equivalent nylon filter
- Vacuum pump
- Knitted reaction coil, 375 µL (P/N 043700)

For Sample Preparation

- Syringe filters, 25 mm, 0.2 µm, IC Certified (Pall Corporation, IC Acrodisc®, Supor® PES, P/N 4583)
- If available, mechanical wrist agitator for free glycerol determinations

For Total Glycerol Determinations

- Reflux distillation apparatus with a cooling condenser, a thermometer, and two or more 125 mL distillation flasks
- Hot plate/magnetic stirrer with a 1 in. stir bar
- Mineral oil (VWR, P/N AAA31911) bath to moderate the temperature (190 mm diameter crystallizing dish or similar, VWR P/N 89000-298)

Reagents and Standards

- Deionized water, Type 1 reagent-grade, 18.2 MΩ-cm resistivity
- Use only ACS reagent-grade chemicals for all reagents and standards.
- Glycerol (VWR International, JTM778)
- Potassium hydroxide, Baker Analyzed® 45.0% (w/w) (VWR International, P/N JT3143)
- Sodium hydroxide, 50% (w/w) certified (Fisher Chemicals, P/N SS254-500)
- pH 7 (yellow) buffer solution (VWR International, P/N BDH5046)
- pH 10 (blue) buffer solution (VWR International, P/N BDH5072)
- Methanol or isopropanol for cleaning the distillation flasks

Samples

- Ten biodiesel samples:
  - B100: soy, animal, corn, animal/vegetable
  - B6: soy
  - B10: animal, soy, corn, animal/vegetable
  - B20: soy

Diesel sample: B0 Ultra-Low Sulfur Diesel (ULSD)

Conditions	
Column:	Dionex CarboPac MA1 Guard, 4 × 50 mm (P/N 044067) Dionex CarboPac MA1 Analytical, 4 × 250 mm (P/N 044066)
Eluent A:	Degassed deionized water
Eluent B:	500 mM Sodium hydroxide
Eluent:	100 mM Sodium hydroxide (20% Eluent B)
Flow Rate:	0.40 mL/min
Column Temp.:	30 °C
Inj. Volume:	5 µL
Detection:	PAD, certified carbohydrate Au working electrode, four-potential carbohydrate waveform (Waveform A)[7,9] see Table 1
Frequency:	2 Hz
Typical Background:	15–25 nC
Typical Noise:	10–20 pC
Typical System Backpressure:	1300 psi
Typical pH:	12.3–12.5
Run time:	15 min

Table 1. Waveform A, four-potential carbohydrate waveform.[9]

Time (s)	Potential vs Ag/AgCl (V)	Gain Region[a]	Integration	Ramp[a]
0.00	+ 0.10	Off	Off	Ramp
0.20	+ 0.10	On	On (Start)	Ramp
0.40	+ 0.10	Off	Off (End)	Ramp
0.41	– 2.00	Off	Off	Ramp
0.42	– 2.00	Off	Off	Ramp
0.43	+ 0.60	Off	Off	Ramp
0.44	– 0.10	Off	Off	Ramp
0.50	– 0.10	Off	Off	Ramp

[a]The gain and ramp are instrument settings for the Dionex ICS-3000 electrochemical detector.

Preparation of Solutions and Reagents

General Tips

Rinse glassware, pipettes, sample vials, and sample containers with deionized water prior to the handling or introduction of the samples to minimize contamination. Include a control blank to determine the baseline contamination from the sample handling and sample preparation processes.

Eluent Solutions

When preparing eluents, it is essential to use high-quality, Type 1, 18.2 MΩ-cm resistivity deionized water that contains as little dissolved carbon dioxide as possible. Carbon dioxide dissolved in water and hydroxide eluents produces carbonate, which can compromise the chromatography. In addition, dissolved gases also cause increased noise. Degas the deionized water before eluent preparation by using vacuum filtration and ultrasonic agitation with applied vacuum for 10 to 20 min (Dionex [now part of Thermo Scientific] Technical Note 71).[10] Additionally, prepare 1 L of degassed Type 1 water weekly for the AS Autosampler flush solution.

Eluent A Solution (Degassed Deionized Water)

Prepare 2 L of degassed deionized water as described above. Connect the eluent bottle to the Eluent A line from the pump and apply ~4–5 psi of head pressure using nitrogen or another inert gas and prime the pump with the new eluent.

Eluent B Solution (500 mM Sodium Hydroxide)

It is essential that high-purity (Fisher) 50% (w/w) NaOH is used for eluent preparation. Do not use NaOH pellets because they are coated with sodium carbonate, as the carbonate will bind to the column and interfere with the column selectivity, resolution, and peak efficiency.[10]

To prepare 2 L of 500 mM NaOH, add 1947.6 g of degassed deionized water into a 2 L precleaned eluent bottle on a top loader balance that is accurate to ± 0.01 g. Do not use glass bottles with hydroxide eluents as this can release compounds that foul the working electrode. Position a 25 mL transfer pipette in the center of the bottle and transfer 80 g (52.4 mL) of 50% (w/w) NaOH solution to the 2 L eluent bottle. Immediately close and cap the hydroxide and eluent bottles. Connect the eluent bottle to the Eluent B line from the pump and place the bottle under ~4-5 psi of head pressure using nitrogen or another inert gas. Swirl the eluent bottle to thoroughly mix the eluent and then prime the pump. For additional information on eluent preparation, refer to Technical Note 71.[10]

Sodium Hydroxide for System Decontamination

Prepare 1 L of 2 M NaOH in a similar way as the 500 mM NaOH eluent using 160 g (105 mL) of 50% (w/w) sodium hydroxide solution and 895 g of degassed deionized water into a 1 L precleaned eluent bottle. Connect the eluent bottle to the Eluent C line from the pump and place the bottle under ~4–5 psi of head pressure using nitrogen or another inert gas. Swirl the eluent bottle to thoroughly mix the eluent and then prime the pump.

Potassium Hydroxide Hydrolysis Solution for Total Glycerol Determinations

Use ACS-grade purity 45% (w/w) potassium hydroxide reagent for this solution to prevent the introduction of contaminants. To prepare 1 L of 1 M potassium hydroxide solution, add 200 mL deionized water into a 1 L HDPE volumetric flask placed on a top loader balance that is accurate to ± 0.01 g. Tare the balance and transfer 124.7 g (86.3 mL) of 45.0% (w/w) potassium hydroxide solution with a pre-rinsed 25 mL plastic transfer pipette into the volumetric flask. Remove the flask, add deionized water to the 1 L mark, and mix thoroughly. Transfer the 1 M potassium hydroxide solution to 1 L HDPE bottle. Prepare a fresh solution weekly.

Standards

Stock Standard Solution

To prepare 1000 mg/kg of glycerol stock solution, weigh 1.000 g of the > 99.5% glycerol solution into a 1 L glass volumetric flask placed on an analytical balance. Add ~200 mL of deionized water and mix thoroughly using a vortex mixer. Dilute to the 1 L mark with deionized water. Insert stopper, shake, and invert the flask to thoroughly mix the standard stock solution. Prepare the stock solution monthly and store at 5 °C.

Working Standard Solutions

To prepare 0.5, 1.0, 5.0, 10.0, 20.0, and 50.0 mg/kg glycerol working standard solutions, add an appropriate volume of the 1000 mg/kg glycerol stock standard in separate 100 mL glass volumetric flasks measured on an analytical balance. Dilute the working solutions to the 100 mL mark and mix the solutions with the vortex mixer. Prepare the working standards daily and store at 5 °C. Prepare the LOD and LOQ standards from serial dilutions of the 0.5 mg/kg working standard.

Sample Preparation

Mix the neat sample to a homogenous appearance prior to removing an aliquot for sample analysis.

Free Glycerol Determinations

Weigh 5.000 g of sample into a 125 mL HDPE bottle, add 50.000 g of deionized water, and record the weights of the sample and the water. Shake each bottle manually or with a mechanical wrist agitator for 5 min. Allow the aqueous layer to separate from the organic layer. Transfer the aqueous layer to a 20 mL glass scintillation vial. Additionally, filter the aqueous layers of each sample with an IC Acrodisc (0.2 µm) syringe filter prior to analysis to protect the columns, and dilute the samples appropriately for the calibration range.

Total Glycerol Determinations

Sample Hydrolysis

To assemble the reflux distillation apparatus, position the crystallizing dish containing mineral oil on the hot plate/magnetic stirrer in an exhaust hood. Then place the distillation flask in the mineral oil and assemble the distillation apparatus with a cooling water condenser in the reflux configuration, i.e., vertically. Connect the

cooling water to the condenser and the drain line from the condenser to the drain. Note: These are only general instructions for setting up a reflux distillation apparatus. Carefully review the set-up of the apparatus used and consult with your safety officer before performing this sample preparation.

To prepare the samples, weigh 2.500 g of sample into a 125 mL distillation flask on an analytical balance. Remove the flask from the balance to the exhaust hood. Transfer 20 mL of the 1 M potassium hydroxide solution with a pre-rinsed plastic 25 mL transfer pipette into the distillation flask containing the sample and add a magnetic stir bar for stirring. Install the flask into the mineral oil bath and reflux distillation apparatus. Then, reflux the sample solution with continuous stirring at 95 °C for 1 h. When the reflux is complete, remove the distillation flask, and cool to room temperature. This allows the refluxed sample to separate into aqueous and organic layers. Wipe the excess mineral oil from the distillation flask and transfer the aqueous layer using a transfer pipette into a tared 100 mL sample container. The Corn B100 biodiesel sample forms a homogenous viscous syrup that can not be transferred by pipette. Therefore, the whole sample must be poured into a tared container.

For all samples, rinse the distillation flask with three 10 mL increments of deionized water and transfer each aliquot to the tared sample container that contains the original aqueous layer (~20 g) from the refluxed sample. The final sample weight must be 50.000 g. Mix the aqueous solution on a vortex mixer to a homogenous appearance. Additionally, filter the aqueous layer of each sample with a syringe filter prior to analysis to protect the columns and dilute the samples appropriately for the calibration range.

To prepare for the next sample, discard the remaining solvent layer from the distillation flask into a solvent waste container and rinse the flask with methanol or isopropanol until clean. Repeat the reflux process for the next sample.

Spike Recovery Samples

Prepare a 100 mg/kg spiking standard solution for free glycerol determinations by adding 10.000 g of the 1000 mg/kg stock standard to 100 mL glass volumetric flask and diluting to volume with deionized water. Mix thoroughly. To prepare the spiked solutions, spike the samples with 10–100 µL of the 100 mg/kg spiking solution prior to water extraction. Treat the spiked samples the same as the unspiked samples as described for free glycerol determinations. Spike the filtered, final refluxed samples for total glycerol determinations using the same spiking solutions. Dilute the samples appropriately for the calibration range.

System Preparation and Setup

Configuring Virtual Channel to Monitor pH

The continuous monitoring of pH during sample analyses provides details on reference electrode drift and noise, and is one measurement to confirm proper eluent preparation. To monitor the pH, follow the instructions in Dionex (now part of Thermo Scientific) Application Note (AN) 188 to create a Virtual Channel using the Server Configuration program.[11] After the virtual channel is configured, the pH virtual channel becomes one of the available signal channels.

General Tips

As a precaution to minimize microbial contamination, temporarily install a 1000 psi backpressure loop between the pump and the injection valve and pump 2 M NaOH through the eluent lines at 0.5 mL/min for 1 to 2 h followed by 100 mM NaOH eluent for another 1 to 2 h.[12] Remove the backpressure loop prior to installing the column.

Glass sample vials are recommended when determining free glycerol because of the lower possibility of contamination. However, polypropylene sample vials were used here to determine total glycerol because the sample contains ~0.5 M potassium hydroxide that can etch the glass and foul the working electrode. Both types of vials must be rinsed multiple times with deionized water prior to use.

Preparing a 5 µL Sample Loop

To prepare a 5 µL sample loop, cut 10 to 12 cm length of black PEEK™ tubing. Weigh the tubing on an analytical balance before and after filling the tubing with deionized water. Adjust the length until the net weight is 5 ± 1 mg (equivalent to 5 ± 1 µL) with three consecutive measurements. Record the average calibrated weight into the AS Autosampler module and install the sample loop in the injection valve.

Plumbing the Chromatography System

To plumb the system for glycerol determinations, install black PEEK tubing between the pump and injection valve, and red PEEK tubing for all other eluent lines after the injection valve to the cell inlet. Follow the instructions in the product manual to install the Dionex CarboPac MA1 column set.[13] Once installed, the expected system backpressure of the Dionex CarboPac MA1 column set is typically ~1300 psi. Allow the column to equilibrate overnight using the method conditions.

Assembling the Electrochemical Cell

To assemble the electrochemical cell, follow the instructions in AN 188, calibrate the reference electrode from pH 7 to 10, and install a disposable certified carbohydrate Au working electrode. The disposable Au working electrode has a typical lifetime of two weeks in a strong base eluent using the four-potential carbohydrate waveform (Waveform A). Typically, the background of a system using a new Au disposable working electrode will stabilize within 10 min. For more information, refer to the disposable electrode manual.[14]

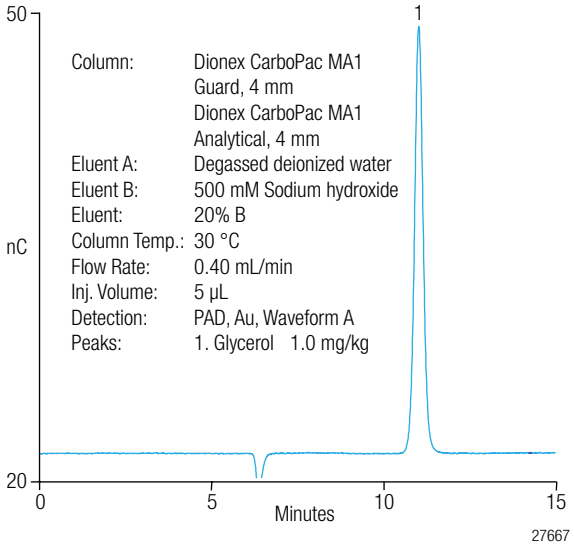

Figure 1. Glycerol determined by HPAE-PAD.

Results and Discussion

Glycerol was separated on a Dionex CarboPac MA1 (4 × 250 mm) column using 100 mM sodium hydroxide and detected with PAD and a disposable Au working electrode. This method takes advantage of the high capacity (1.45 mEq) and selectivity of the Dionex CarboPac MA1 column to improve the retention of glycerol relative to other CarboPac columns. The four-potential waveform is a well-established waveform optimized to provide a stable response when determining carbohydrates.[7,9] Figure 1 shows the separation of 1.0 mg/kg glycerol using this method. The chromatogram shows a symmetrical glycerol peak (A_s = 1.03, EP)[a] eluting from the column at 10.9 min with a peak response of 25.9 nC, which is well above the baseline.

[a] A_s (EP) is defined as the peak widths of the right half and left half of the peak divided by two times the width of the left half of the peak, where all peak widths are measured at 5% of the peak height.

Limit of Detection, Limit of Quantification, Linear Range, and Precision

To qualify the method, the estimated limit of detection (LOD), limit of quantification (LOQ), linear range, and precision were determined. The LOD and LOQ were determined by measuring the peak-to-peak noise in 1 min increments from 20 to 60 min in four replicate runs without a sample injection. The noise averaged 13 ± 1.9 pC. The estimated LOD and LOQ of glycerol were calculated as 0.5 and 2.4 µg/kg, respectively based on the peak response of the standard at 3× and 10× the signal-to-noise (S/N). To determine the method linearity, seven glycerol calibration standards from 0.05 to 10 mg/kg in deionized water were injected. The calibration results were linear with a correlation coefficient (r^2) of 0.9998. The retention time and peak area precisions, based on seven replicate injections of a 0.7 mg/kg glycerol standard, had RSDs of 0.04 and 0.89, respectively.

Table 2. Free and total glycerol concentrations in biodiesel samples.[a]

Sample	Free Glycerol		Total Glycerol	
	(mg/kg)	(Wt %)[b]	(mg/kg)	(Wt %)[b]
B0 (ULSD Diesel)	0.021	0.00	0.069	0.00
B0 (ULSD Diesel) Duplicate	0.0226	0.00	0.064	0.00
B100-1 (Soy)	1.12	0.00	200	0.20
B100-1 (Soy) Duplicate	1.19	0.00	199	0.20
Blend 1 (Soy B6)	0.061	6.0×10^{-5}	11.8	0.01178
Blend 1 (Soy B6) Duplicate	0.073	7.0×10^{-5}	11.8	0.01182
Blend 2 (Soy B10)	0.09	9.0×10^{-5}	19.0	0.01899
Blend 2 (Soy B10) Duplicate	0.1991	1.0×10^{-4}	19.4	0.01938
Blend 3 (Soy B20)	0.216	2.2×10^{-4}	20.4	0.02043
Blend 3 (Soy B20) Duplicate	0.233	2.3×10^{-4}	20.6	0.02065
B100-2 (Animal)	5.34	0.01	193	0.19
B100-2 (Animal) Duplicate	5.08	0.01	188	0.19
Blend 4 (Animal B10)	0.16	1.6×10^{-4}	7.52	0.00752
Blend 4 (Animal B10) Duplicate	0.16	1.6×10^{-4}	7.35	0.00735
B100-3 (Animal/ Vegetable)	6.98	0.01	191	0.19
B100-3 (Animal/ Vegetable) Duplicate	6.56	0.01	185	0.19
Blend 5 (Animal/ Vegetable B10)	0.233	2.3×10^{-4}	16.8	0.01676
Blend 5 (Animal/ Vegetable B10) Duplicate	0.24	2.4×10^{-4}	16.3	0.01632
B100 Corn	11.6	0.01	140	0.14
B100 Corn Duplicate	12.0	0.01	143	0.14
Blend 6 (Corn B10)	0.37	3.7×10^{-4}	12.5	0.01249
Blend 6 (Corn B10) Duplicate	0.426	4.3×10^{-4}	12.9	0.01291

[a]Duplicate samples are separate sample preparations.
[b]ASTM reporting requirements to X.XX and X.XXXXX wt % for B100 and blends, respectively.

Glycerol in Biodiesel Samples

Calculations

The results of glycerol determinations were reported to the ASTM Inter-Laboratory Study (ILS) 457 Collaboration Committee as percent free and total glycerol by multiplying the glycerol concentration in mg/kg by the dilution factor and a conversion factor of 10,000 (Table 2).

Column:	Dionex CarboPac MA1 Guard, 4 mm Dionex CarboPac MA1 Analytical, 4 mm
Eluent A:	Degassed deionized water
Eluent B:	500 mM Sodium hydroxide
Eluent:	20% B
Column Temp.:	30 °C
Flow Rate:	0.40 mL/min
Inj. Volume:	5 µL
Detection:	PAD, Au, Waveform A
Sample Prep.:	Water extraction, 5 min agitation, 0.2 µm
Samples:	A) B100 Soy B) B100 Soy + 1.2 mg/kg glycerol

Peaks:	A	B	
1. Unknown	–	–	mg/kg
2. Glycerol	1.1	2.2	

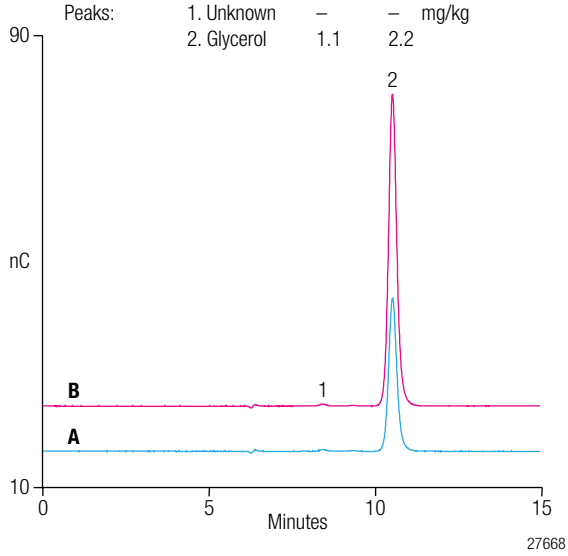

Figure 2. Comparison of free glycerol in separate extractions of a B100 soy sample A) without and B) with added glycerol.

Column:	Dionex CarboPac MA1 Guard, 4 mm Dionex CarboPac MA1 Analytical, 4 mm
Eluent A:	Degassed deionized water
Eluent B:	500 mM Sodium hydroxide
Eluent:	20% B
Column Temp.:	30 °C
Flow Rate:	0.40 mL/min
Inj. Volume:	5 µL
Detection:	PAD, Au, Waveform A
Sample Prep.:	Base-hydrolysis, aqueouslayer: 0.2 µm, dilute 1:100
Samples:	A) B100 Animal B) Sample A + 0.80 mg/kg glycerol

Peaks:	A	B	
1. Unknown	–	–	mg/kg
2. Glycerol	0.54	1.3	

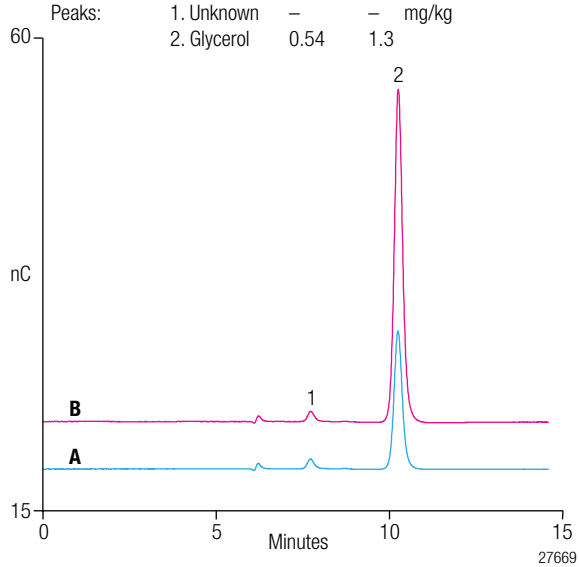

Figure 3. Comparison of total glycerol in an aqueous solution of a base-hydrolyzed B100 animal sample A) without and B) with added glycerol.

Dilution factor (dF) = [(weight (g) of final solution) / (sample weight (g)] × (dilution of sample for analysis)

Final concentration (wt %) = [measured concentration (mg/kg) × dF] / (10,000 conversion factor)

Duplicate samples were prepared for both free and total glycerol determinations for all samples. Table 2 shows the results in weight % and in mg/kg concentration units. The free glycerol results ranged from 0.02 mg/kg for B0 ULSD diesel to 12.0 mg/kg for B100 corn (0.00 to 0.01 wt %). The total glycerol results ranged from 0.064 mg/kg for B0 ULSD diesel to 200 mg/kg for the B100 soy, B100 animal, and B100 animal/vegetable (0.00 to 0.20 wt %) samples. Figure 2A shows a chromatogram of free glycerol in the B100 soy sample by HPAE-PAD. The glycerol peak had a good symmetry of 1.1 (A_s EP) and was well resolved from an unknown peak [R_s = 3 (EP)][b], which was likely ethylene glycol.

[b] R_s (EP) is defined as 1.18 times the difference of the retention times of the reference peak and peak of interest, divided by the sum of both peak widths measured at 50% peak height.

Figure 3A shows a chromatogram of a 1:100 dilution of total glycerol in the B100 animal sample. The glycerol peak has the same symmetry of 1.1 (A_s EP) and was well resolved with an R_s of 6 (EP) from a different unknown peak, which was likely propylene glycol. Total glycerol concentrations had 1–2 orders of magnitude higher concentrations than free glycerol, as expected. The free and total glycerol concentrations of all biodiesel samples were within the ASTM specifications of <0.02 and <0.24 wt %, respectively.

Accuracy and Precision

Retention time precisions for glycerol were determined from seven replicate injections, which produced <0.1 RSDs for all samples (not shown). The peak area precisions were determined from the same set of injections with RSDs from 0.27–5.64 (Table 3). Accuracy was determined by the recovery of glycerol spiked into the samples. In the free glycerol samples, glycerol was spiked into the 50.000 g of water used to extract free glycerol. Conversely, in the total glycerol samples, glycerol was added to the filtered, aqueous layer of the base-hydrolyzed samples (Tables 4–5). The recoveries ranged from 93.9 to 104.4% and 92.1 to 100.0% for free and total glycerol samples, respectively. Figure 2 demonstrates the chromatography of a free glycerol sample from separate extractions of a B100 soy biodiesel sample without and with glycerol added. Similarly, Figure 3 demonstrates the chromatography for total glycerol of a B100 animal biodiesel sample, diluted 1:100, without and with glycerol added.

Table 3. Peak area precisions for free and total glycerol determinations.

	Free Glycerol (nC-min)	RSD	Total Glycerol (nC-min)	RSD
ULSD B0	0.100 ± 0.001	5.64	0.243 ± 0.006	2.5
Soy B100-1	8.33 ± 0.02	0.20	73.4 ± 0.6[c]	0.78
Soy B6	0.374 ± 0.007	1.93	8.94 ± 0.05[b]	0.61
Soy B10	0.607 ± 0.011	1.79	14.4 ± 0.2[b]	1.2
Soy B20	1.55 ± 0.01	0.65	15.7 ± 0.1[b]	0.36
Animal B100-2	24.6 ± 0.5	2.23	12.6 ± 0.1[d]	1.1
Animal B10	1.14 ± 0.01	0.71	5.54 ± 0.3[b]	0.60
Animal/Vegetable B100-3	52.3 ± 0.1	0.27	14.5 ± 0.0[d]	0.97
Animal/Vegetable B10	1.38 ± 0.01	0.96	12.6 ± 0.1[b]	0.97
Corn B100	28.8 ± 0.3[a]	1.03	11.9 ± 0.3[d]	0.25
Corn B10	2.68 ± 0.02	0.69	4.81 ± 0.04[c]	0.73

n = 7
Samples were diluted [a]1:3, [b]1:5, [c]1:10, and [d]1:50 prior to glycerol determinations.

Table 4. Recovery of free glycerol from aqueous extractions of biodiesel samples.[a]

	Free Glycerol Present (mg/kg)	Added (mg/kg)	Free Glycerol Found (mg/kg)	Recovery (%)
ULSD B0	0.032 ± 0.001	0.100	0.133 ± 0.001	102.0
Soy B100-1	1.08 ± 0.00	1.20	2.21 ± 0.016	96.8
Soy B6	0.043 ± 0.001	0.100	0.142 ± 0.001	99.4
Soy B10	0.070 ± 0.003	0.100	0.167 ± 0.001	98.0
Soy B20	0.184 ± 0.009	0.100	0.297 ± 0.005	104.4
Animal B100-2	3.22 ± 0.29	4.00	7.65 ± 0.24	101.7
Animal B10	0.144 ± 0.002	0.240	0.386 ± 0.01	102.0
Animal/Vegetable B100-3, 1:3 dilution	2.13 ± 0.03	4.0	5.92 ± 0.06	96.6
Animal/Vegetable B10	0.232 ± 0.002	0.240	0.454 ± 0.009	96.2
Corn B100, 1:5 dilution	2.11 ± 0.00	2.40	4.48 ± 0.00	100.8
Corn B10	0.387 ± 0.014	0.100	0.455 ± 0.001	93.9

n = 3

[a]Samples were spiked prior to water extraction used to determine free glycerol.

Table 5. Recovery of glycerol added to aqueous aolutions of base-hydrolyzed biodiesel samples used for total glycerol determinations.[a]

	Total Glycerol Present (mg/kg)	Added (mg/kg)	Total Glycerol Found (mg/kg)	Recovery (%)
ULSD B0	0.059 ± 0.003	0.124	0.183 ± 0.003	100
Soy B100-1[b]	3.99 ± 0.01	4.98	8.61 ± 0.02	96.6
Soy B6[c]	1.09 ± 0.00	1.21	2.12 ± 0.02	92.2
Soy B10[c]	1.73 ± 0.02	2.00	3.45 ± 0.05	92.6
Soy B20[c]	3.42 ± 0.04	1.93	5.03 ± 0.02	93.9
Animal B100-2[d]	0.535 ± 0.002	0.798	1.27 ± 0.00	95.3
Animal B10[c]	1.21 ± 0.00	0.607	1.74 ± 0.00	95.5
Animal/Vegetable B100-3[d]	1.68 ± 0.00	2.01	3.40 ± 0.01	92.1
Animal/Vegetable B10[c]	1.53 ± 0.01	2.01	3.46 ± 0.01	97.8
Corn B100[e]	1.31 ± 0.00	1.60	2.83 ± 0.01	97.1
Corn B10[c]	1.18 ± 0.01	1.60	2.64 ± 0.01	95.0

n = 3
[a]Samples were spiked into the diluted aqueous layer of the base-hydrolyzed biodiesel samples.
Samples were further diluted [b]1:20, [c]1:5, [d]1:50, and [e]1:100 for analysis.

Conclusion

This application describes an HPAE-PAD method to accurately and selectively determine free and total glycerol from aqueous extractions of biodiesel samples and base-hydrolyzed biodiesel samples, respectively. HPAE-PAD is a sensitive and selective method that does not require sample derivatization, and therefore is the method of choice when determining µg/kg to mg/kg concentrations of carbohydrates.

List of Suppliers

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Fisher Chemicals, part of Thermo Fisher Scientific, 2000 Park Lane Drive, Pittsburgh, PA 15275.
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VWR International, Inc., 1310 Goshen Parkway, West Chester, PA, USA 19380. Tel: 1-800-932-5000
www.vwrsp.com

References

1. International Panel for Sustainable Resource Management, United Nations Environmental Programme (UNEP), Division of Technology, Industry and Economics (UNEP-DTIE). *Towards Sustainable Production and Use of Resources: Assessing Biofuels*. UNEP-DTIE, Paris, 2009.
2. Evans, G. *Liquid Transport Biofuels–Technology Status Report, International Biofuels Strategy Project,* The National Non-Food Crops Centre, York, UK, 2007.
3. Meher, L. C.; Sagar, D. V.; Naik, S. N. Technical Aspects of Biodiesel Production by Transesterication—a Review. *Ren. Sustainable Energy Rev.* **2006,** *10,* 248–268.
4. Ma, F.; Hanna, M.A. Biodiesel Production: a Review. *Bioresour. Technol.* **1999,** *70,* 1–15.
5. McCormick, R. L.; Westbrook, S. R. Biodiesel and Biodiesel Blends. *ASTM Standardization News* April **2007.**
6. ASTM International. ASTM D 6751, Specification for Biodiesel Fuel Blend Stock (B100) for Middle Distillate Fuels. West Conshohocken, PA, 2008.
7. Rocklin, R. D.; Clarke, A. P.; Weitzhandler, M. Improved Long-Term Reproducibility for Pulsed Amperometric Detection of Carbohydrates via a new Quadruple-Potential Waveform. *Anal. Chem.* **1998,** *70* (8), 1496–1501.
8. Hanko, V.; Rohrer, J. Determination of Carbohydrates, Sugar Alcohols, and Glycols in Cell Cultures and Fermentation Broths using High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection. *Anal. Biochem.* **2000,** *283,* 192–199.
9. Dionex Corporation (now part of Thermo Scientific). *Optimal Settings for Pulsed Amperometric Detection of Carbohydrates using the Dionex ED40 Electrochemical Detector.* Technical Note 21, LPN 034889, Sunnyvale, CA, 1998.
10. Dionex Corporation (now part of Thermo Scientific). *Eluent Preparation for High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection.* Technical Note 71, LPN 1932, Sunnyvale, CA, 2009.
11. Dionex Corporation (now part of Thermo Scientific). *Determination of Glycols and Alcohols in Fermentation Broths by Ion-Exclusion Chromatography and Pulsed Amperometric Detection*. Application Note 188, LPN 1944, Sunnyvale, CA, 2008.
12. Dionex Corporation (now part of Thermo Scientific). *Analysis of Carbohydrates by High-Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAE-PAD).* Technical Note 20, LPN 032857, Sunnyvale, CA, 2000.
13. Dionex Corporation (now part of Thermo Scientific). Product Manual for CarboPac MA1, PA1, PA10, PA100 Columns, Document No. 031824. Sunnyvale, CA, 2009.
14. Dionex Corporation (now part of Thermo Scientific). Product Manual for Disposable Gold, Disposable Silver, Disposable Platinum and Disposable Carbon Electrodes, Document No. 065040. Sunnyvale, CA, 2009.

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