Analysis of Free and Total Glycerol in B-100 Biodiesel Methyl Esters Using Agilent Select Biodiesel for Glycerides

Application Note

Energy and Fuels

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Introduction

The American Standard, ASTM D 6584 [1], is the standard test method used to determine the free and total glycerol contents in fatty acid methyl esters (FAMEs), typically intended for pure biodiesel or as a blending component for domestic and diesel fuels. Total glycerol content is calculated from the results. The method is suitable for FAME from rapeseed, sunflower, and soybean oils. It is not suitable for FAME produced from or containing lauric oils, such as coconut and palm kernel oils, due to the problem of peak overlap.

The high performance Agilent Select Biodiesel for Glycerides metal capillary (UltiMetal) GC column was specifically developed for high temperature methods. This column will not break during extreme oven conditions and is produced with a pre-installed retention gap, which provides the performance and robustness required to run this application for an extended period of time.

Biodiesel is produced by transesterifying the parent oil or fat with an alcohol, usually methanol, in the presence of a catalyst, usually a strong base such as sodium or potassium hydroxide, or preferably and increasingly more commonly, alkoxides. The resulting product can contain not only the desired alkyl ester product but also unreacted starting material (TAG, triacylglycerides), residual alcohol, and residual catalysts. Glycerol is formed as a by-product and is separated from biodiesel in the production process. However, traces of glycerol can be found in the final biodiesel product. Since transesterification is a stepwise process, MAG (monoacylglycerides) and DAG (diacylglycerides) formed as intermediates can also be found in biodiesel [2].

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Experimental

Calculation of free and total glycerol

First, the free glycerol (G) and residual mono- (M), di- (D), and triglyceride (T) contents in FAME is determined. The total and bound glycerol content is calculated from the results using the following equation.

Total glycerol (GT) = free glycerol (G) + bound glycerol (BG)

Bound glycerol is calculated using the following equation.

Bound glycerol = 0.2591M + 0.1488D + 0.1044T

The detection range for free glycerol is 0.005 to 0.05 mass %. The detection range for total glycerol is 0.05 to 0.5 mass %. In the standard test method ASTM D 6751 [3], requirements for glycerol used as a blend component with diesel fuel oils are < 0.02 mass % for glycerol and < 0.240 mass % total glycerol.

ASTM 6751 and EN-14105 are two of the most commonly used standardized analytical methods for the analysis of biodiesel.

Materials and methods

Reagents

1,2,4-butanetriol, Internal Standard Solution 1, 1 mg/mL pyridine (IS1)

1,2,3-tricaproylglycerol (tricaprin), Internal Standard Solution 2, 8 mg/mL pyridine (IS2)

Reference materials: glycerol, 1-monooleoylglycerol (monoolein), 1,3-dioleolglycerol (diolein), 1,2,3-trioleoylglycerol (triolein) (GLC standard grade)

Monoglyceride mix (monopalmitin, monostearin, and monoolein), 10 mg/mL pyridine

Conditions

Column	Select Biodiesel for Glycerides UltiMetal 0.32 mm x 10 m, 0.1 µm, with retention gap (p/n CP9076)
Injection	Cold on-column (1093), full EFC control, 1 µL, reversed liner
Temperature	100 °C (1 min) to 370 °C at 15 °C/min
Oven	50 °C (1 min) to 180 °C at 15 °C/min to 230 °C at 7 °C/min to 380 °C (10 min) at 30 °C/min
Carrier gas	Helium, constant flow rate 3 mL/min
Detector	FID, full EFC control, 380 °C

Sample preparation

Different biodiesel samples were obtained from several sources, including in-house prepared biodiesel. This sample was made from rapeseed oil but not at optimized conditions to ensure against a biodiesel sample of suspect quality.

Standard mixtures and internal standard solutions were prepared according to the method. Approximately 100 µL of homogenized biodiesel sample was accurately weighed (± 0.1 mg) in a 20-mL vial, then 100 µL of Internal Standard 1, 100 µL of Internal Standard 2, and 100 µL of MSTFA were added to the sample vials. Care was exercised to ensure there was no contact with any moisture. The vials were hermetically sealed and shaken vigorously. After storing the vials at room temperature for 15 – 20 minutes, approximately 8 mL of heptane was added to each, then 1 µL of the reaction mixture was automatically injected into the gas chromatograph.

Results and Discussion

The method describes the transformation of the glycerol, mono-, and diglycerides into more volatile silylated derivatives in the presence of pyridine and N-methyl-N-trimethylsilyltrifl uoroacetamide (MSTFA). Figure 1 is a chromatogram of a typical B-100 biodiesel sample.

Calibration curves were obtained for glycerol, monoolein, diolein, and triolein. These calibration curves indicate the performance of the system. A typical calibration curve for triolein is shown in Figure 2. Regression coefficients are listed in Table 1.

Based on the calibration curves obtained for glycerol,

Table 1. Regression Coefficients as Calculated by Chromatography Data Station (r^2 must be ≥ 0.99)

Curve y = a x + b	a	b	r^2
Glycerol	1.15585	0.01729	0.9996
Monoolein	1.38085	–0.01468	0.9994
Diolein	1.13841	–0.00772	0.9994
Triolein	0.86610	–0.00720	0.9998

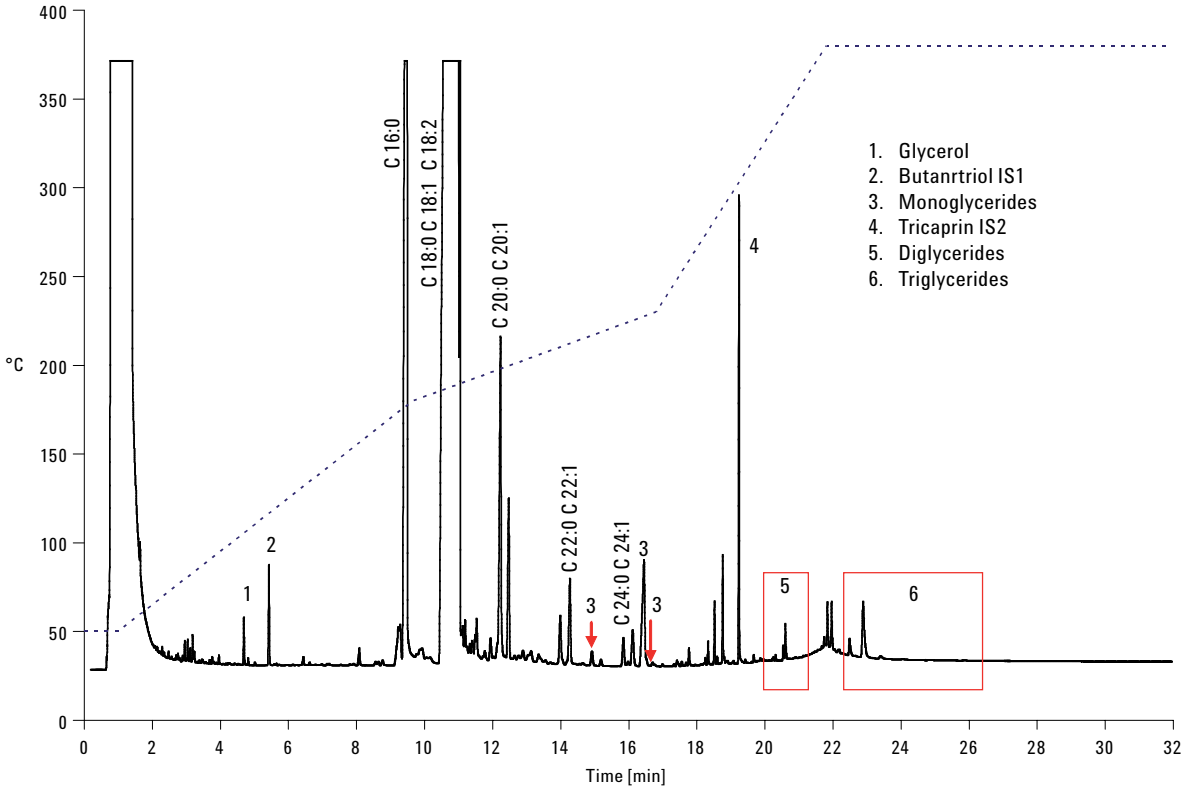

Figure 1. Example chromatogram of a typical B-100 biodiesel sample made from rapeseed oil (with extra glycerol and triglycerides added) after derivatization with MSTFA, analyzed on Agilent Select Biodiesel for Glycerides UltiMetal column. Peaks of interest are separated from the complex matrix, which consists mainly of C18 and C16 FAMEs and minor compounds, such as sterols.

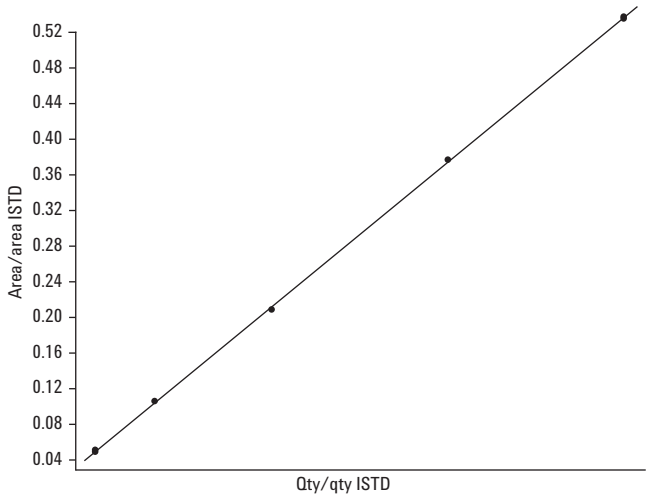

Figure 2. Calibration curve for triolein.

monolein, diolein, and triolein, biodiesel samples were analyzed and quantified: seven regular samples and one spiked sample (code 2, 3S) with extra glycerol (± 0.030 mg) and triglycerides (± 0.48 mg rapeseed oil) per 100 µL. Tables 2 and 3 show typical results for the actual biodiesel samples. Figure 3 depicts the repeatability of the analysis of the spiked sample. The column performance was constant during the analysis of standards and samples (plate number, peak symmetry, and peak width). The variation (expressed as standard deviation) in retention time was typically 0.022 minutes.

A challenging aspect of the method is to accurately integrate the correct peaks using optimized integration parameters. In this example, peak identification was based on a comparison with known standard components. Monoglycerides were integrated from 14.8–15.1 and 16.25–16.8 min, diglycerides from 20.1–20.9 min, and triglycerides from 22.3–26.5 min. The Chromatography Data Station calculates all the peak areas in a specified retention time window and then compares these areas to a calibration curve generated for a single component. See Figures 4, 5 and 6 for details.

Table 2. Typical Analysis Results of a Biodiesel Sample (Winter Biodiesel, Code 1N, 87.9 mg, Duplicate Analysis, Values not Rounded)

Name	Area 1 (µV.min)	Area 2 (µV.min)	Qty average % (m/m)	St. dev.	RSD %
Glycerol	401.6	444.4	0.0109	0.0003	3.08
Butanetriol (IS1 0.08 mg)	10802.7	11319.7	–	–	–
Monoglycerides	2575.4	2482.0	0.6192	0.0046	0.75
Tricaprin (IS2 0.8 mg)	470.3	410.2	–	–	–
Diglycerides	3257.7	3472.2	0.1753	0.0087	4.97
Triglycerides	11743.8	12173.9	0.0463	0.0047	10.2
Bound glycerol	–	–	0.1914	0.0006	0.31
Total glycerides	–	–	0.2023	0.0003	0.12

Table 3. Results of the Free and Total Glycerol Analysis of Biodiesel Samples from Different Origin (Average Mass %, RSD, n = 2)

Code	Description*	Sample mass (mg)	Glycerol	Monoglycerides	Diglycerides	Triglycerides	Bound glycerol	Total glycerides	Meet spec. D6751?
1	Summer biodiesel (2004)	88.8	0.0011^{v} (27%)	0.474 (2.9%)	0.315 (10%)	0.0179 (6.0%)	0.172 (0.7%)	0.173 (0.6%)	Passed
2	Winter biodiesel (3S)	88.6	0.0076 (2.4%)	0.640 (2.1%)	0.139 (2.7%)	0.0657 (7.4%)	0.193 (1.7%)	0.201 (1.7%)	Passed
3	Winter biodiesel (2S)	88.1	0.0014^{v} (31%)	0.644 (3.4%)	0.156 (1.3%)	0.0641 (6.4%)	0.197 (2.5%)	0.198 (2.7%)	Passed
4	Winter biodiesel (1N)	87.9	0.011 (3.1%)	0.619 (0.8%)	0.175 (5.0%)	0.046 (10.2%)	0.191 (0.3%)	0.202 (0.1%)	Passed
5	FAME mix TOFA	89.2	0.0007^{v} (94%)	0.013 (18.3%)	0.049 (4.3%)	0.0075 (0.0%)	0.012 (8.2%)	0.012^{v} (13.1%)	Passed
6	Biodiesel ASTM round robin	87.9	0.0005^{v} (76%)	0.490 (2.3%)	0.111 (2.5%)	0.239 (15.5%)	0.168 (0.8%)	0.169 (0.6%)	Passed
7	In-house prepared biodiesel	89.1	0.0037^{v} (0.6%)	1.743 (1.6%)	8.90 (0.2%)	25.1 (0.6%)	4.39 (1.6%)	4.40^ (1.6%)	Failed

*Descriptions of the biodiesel (B-100) samples are based on the original lab code, time period (Germany, summer or winter biodiesel as sold on the pump) or origin of sample; TOFA: Tall Oil fatty acid methyl ester (mainly C18:1 and C18:2 FAMEs). Sample 2 was analysed with n = 4 (see also Table 2). v = below detection range; ^ = above detection range.

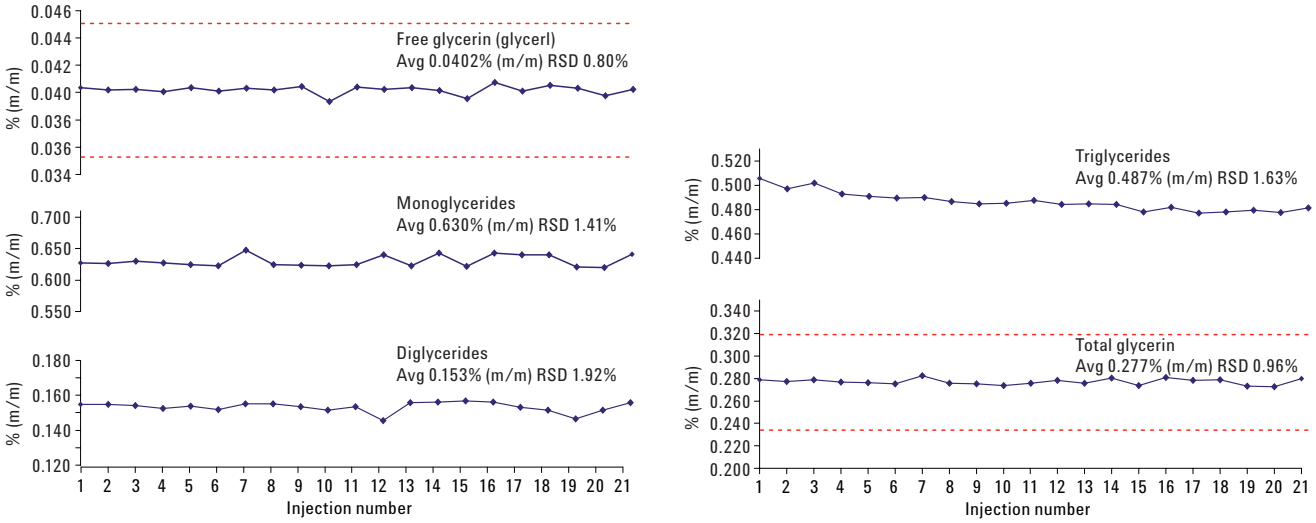

Figure 3. Typical repeatability of 21 successive injections of a spiked biodiesel sample (3S spiked). The red lines represent the maximum variation allowed using the ASTM D 6584 – 07.

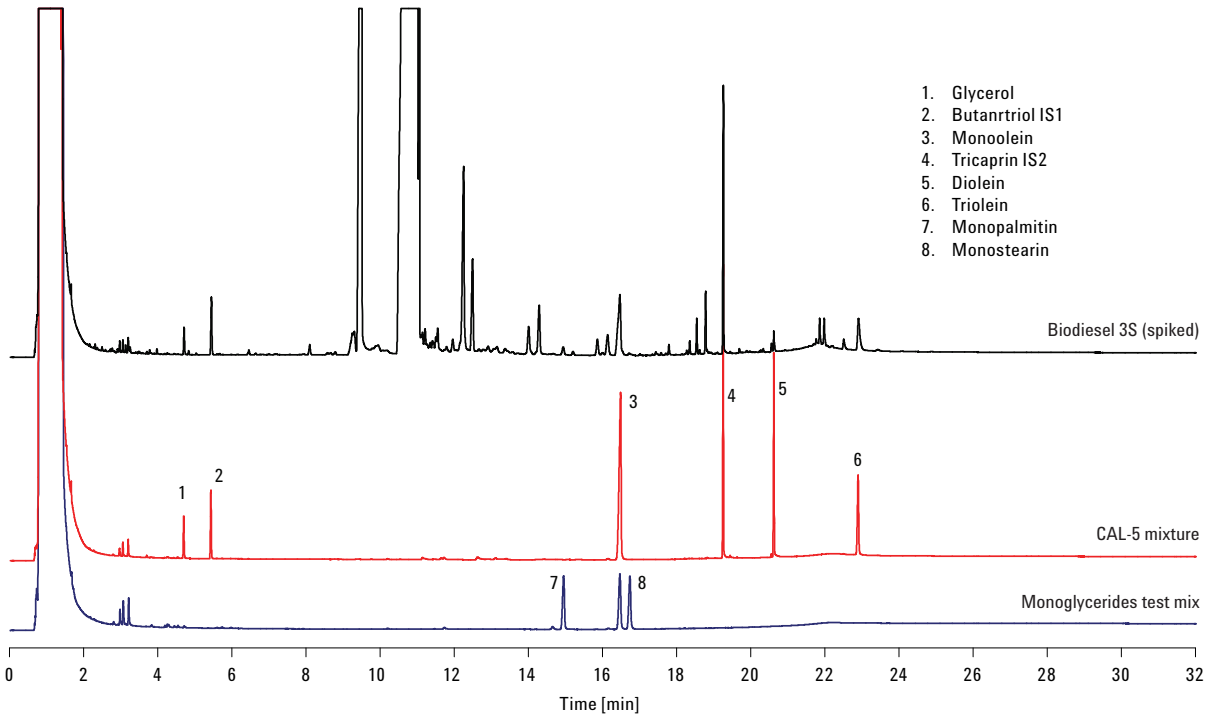

Figure 4. Details of identification of unknown peaks in a biodiesel sample.

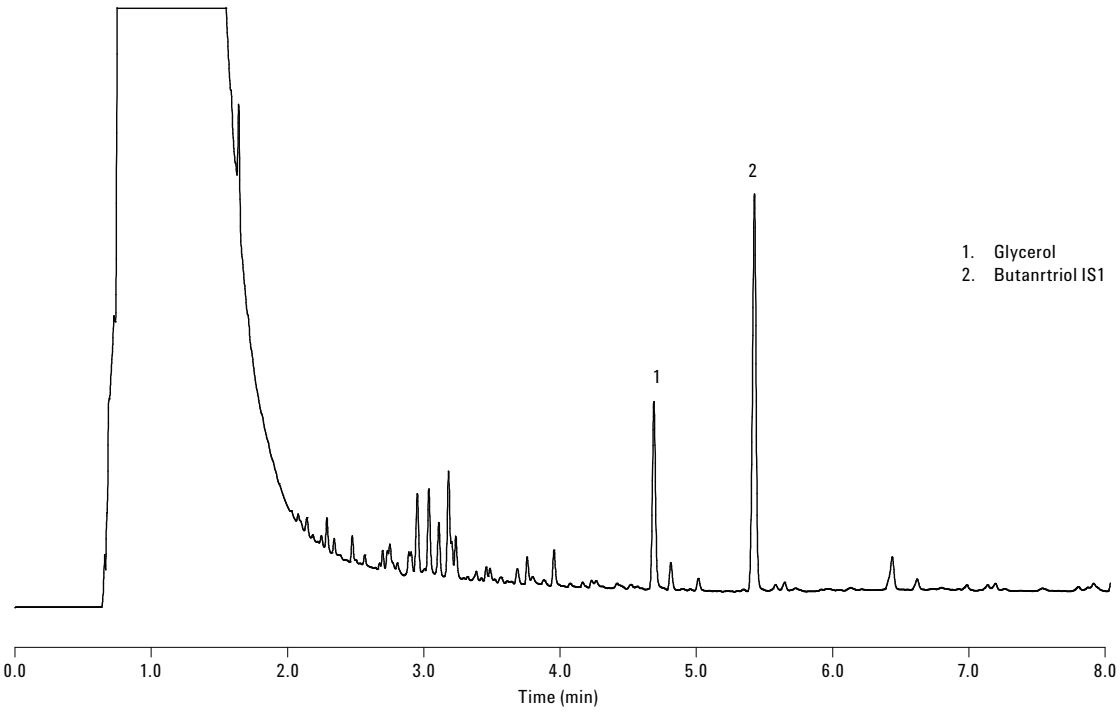

Figure 5. Example chromatogram of B-100 biodiesel (code 3S, with extra glycerol and triglycerides added) with details of the glycerol and internal standard peak.

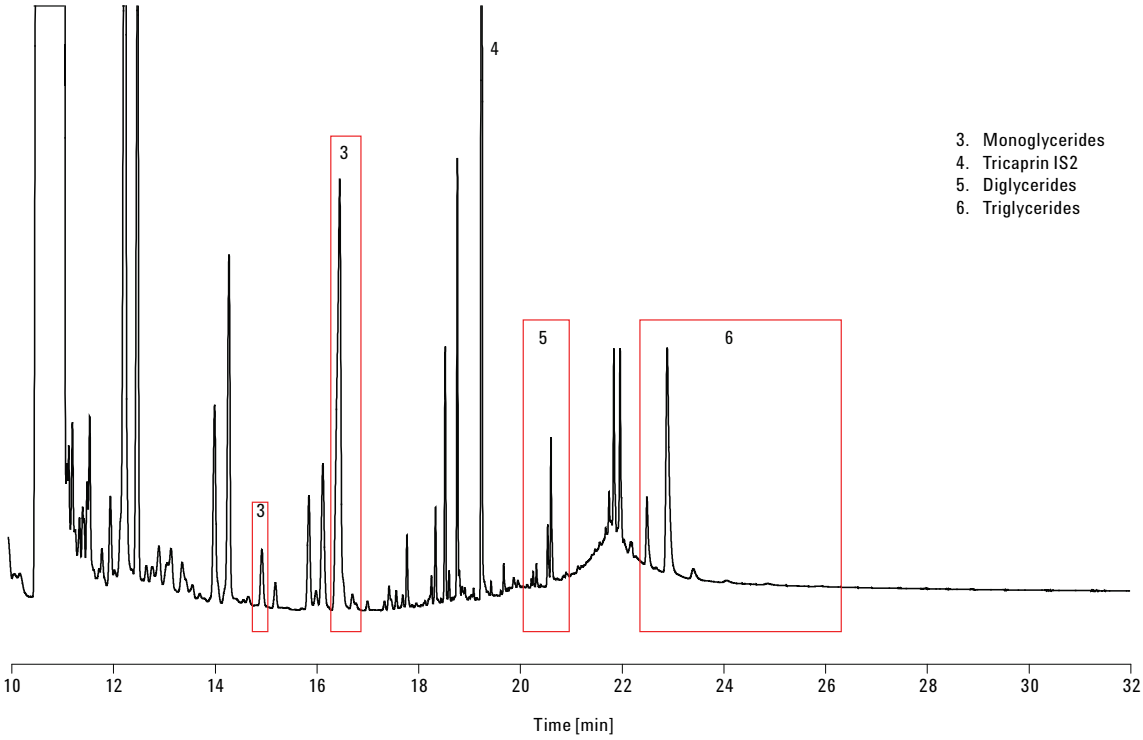

Figure 6. Example chromatogram of B-100 biodiesel (code 3S, with extra glycerol and triglycerides added) with details of the mono-, di-, and triglyceride peak identification and group integration.

It is evident in Figure 6 that there is excellent separation of the triglycerides and very low column bleed at 380 °C. Due to the oven ramp step of 30 °C/min, a broad background peak eluted from the biodiesel matrix at 22 minutes. This had minor effects on the analysis of the di- and triglycerides and is also shown in the ASTM method.

Conclusion

This application note demonstrates the suitability of an on-column injector and the Agilent Select Biodiesel for Glycerides UltiMetal column for the analysis of biodiesel by gas chromatography. The calibration curves and repeatability data demonstrate excellent system integrity, which makes the system ideally suited for the analysis of free, bound, and total glycerol, as well as mono-, di-, and triglyceride content in biodiesel in accordance with ASTM D 6584 [1].

All samples analyzed were within standard specifications as stated in ASTM D 6751 [2] with respect to the maximum level of free glycerol and total glycerol, except for the in-house prepared biodiesel of low quality, which was as expected. However, because of the noted low levels of both glycerol and triglycerides, one sample (3S) was spiked with glycerol and triglycerides so an assessment could be made on the column's separation performance for those components.

The Select Biodiesel for Glycerides column achieves good resolution of biodiesel samples and is a robust solution for this high temperature application. By using an already coupled and tested column from Agilent, problems associated with making a coupling are avoided. In addition, UltiMetal technology removes risks of degradation of the outer coating, and breakage, which makes this column a very robust solution with a long lifetime.

References

1. ASTM D 6584–07. Test Method of Free and Total Glycerine in B-100 Biodiesel Methyl Esters by Gas Chromatography.
2. Knothe, G. (2006), Analyzing Biodiesel: Standards and other Methods. JAOCS 83, 823–833.
3. ASTM D 6751–06a. Standard Specification for Biodiesel Fuel Blend Stock (B100) for Middle Distillate Fuels.

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