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

Application Note

Energy and Fuels

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Introduction

The European Standard, EN-14105 [1], is the standard test method used to determine the free glycerol and residual mono-, di-, and triglyceride 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 preinstalled 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

The method is applicable for a concentration range from 0.005 – 0.05% (m / m) for glycerol (G), 0.25 – 1.25% (m/m) for monoglycerides (M), 0.05 – 0.5% (m/m) for diglycerides (D), and 0.05 – 0.4% (m/m) for triglycerides (T). The total glycerol (GT) content is calculated using the following equation.

$$GT = G + 0.255M + 0.146D + 0.103T$$

In the European standard method EN-14214:2003 [3], requirements for glycerol are 0.02, 0.8, 0.2, 0.2, and 0.25% (m/m). This method and ASTM D 6584 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 × 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 370 °C (5 min) at 10 °C/min
Carrier gas	Helium, constant flow rate 4 mL/min
Detector	FID, full EFC control, 380 °C

Sample preparation

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 diolein is shown in Figure 2. Regression coefficients are listed in Table 1.

Table 1. Regression Coefficients as Calculated by Chromatography Data Station (r^2 must be ≥ 0.95)

Curve y = a x + b	a	b	r^2
Glycerol	1.08243	0.00813	0.9994
Monoolein	1.35878	0.00105	1.0000
Diolein	1.13201	–0.00727	0.9999
Triolein	0.96579	–0.01490	0.9992

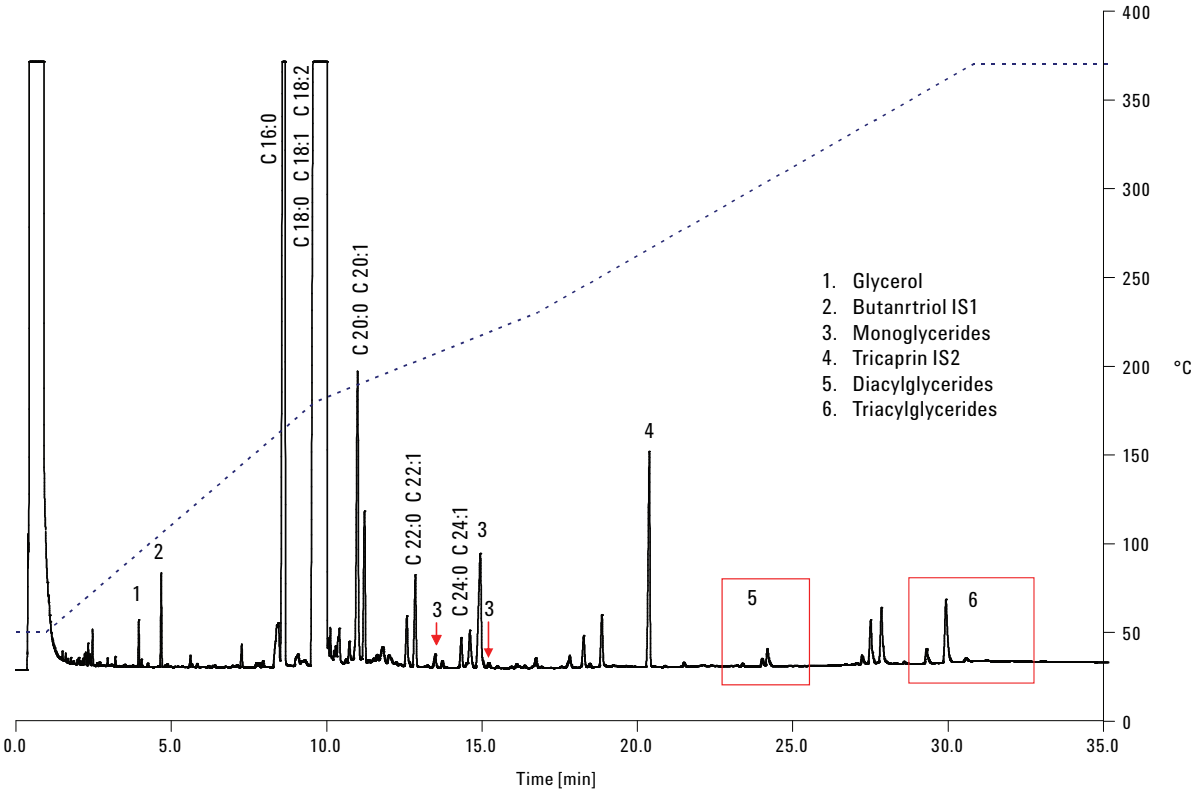

Figure 1. Example chromatogram of a typical B-100 biodiesel sample made from rapeseed oil (with extra glycerol and triglycerides added) after derivatization with MSTFA, analyzed on Agilent Select Biodiesel for Glycerides UltiMetal column. Peaks of interest are separated from the complex matrix, which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

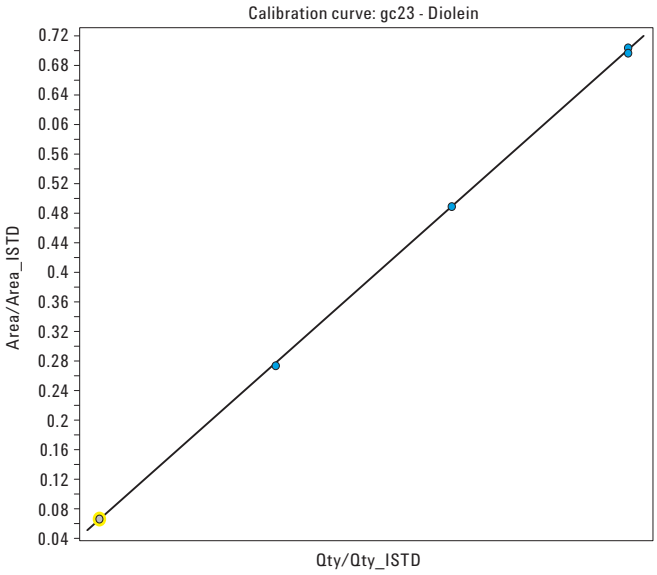

Figure 2. Calibration curve for diolein.

Based on the calibration curves obtained for glycerol, monoolein, diolein, and triolein, two biodiesel samples were analyzed and quantified: a sample with code 3S and the same sample spiked with extra glycerol (± 0.028 mg) and triglycerides (± 0.48 mg rapeseed oil) per 100 µL. Table 2 shows typical results for the actual biodiesel sample. 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 0.005 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 13.15–13.60 and 14.50–15.40 minutes, diglycerides from 22.90–25.50 minutes, and triglycerides from 29–34 minutes. 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, 89.5 mg, Duplicate Analysis, Values not Rounded)

Name	Area 1 (µV.min)	Area 2 (µV.min)	Qty average % (m/m)	St. dev.	RSD %
Glycerol	311.5	307.3	0.0072	0.00010	1.46
Butanetriol (IS1 0.08 mg)	3255.5	3272.7	–	–	–
Monoglycerides	17514.8	17656.2	0.7844	0.00003	0.003
Tricaprin (IS2 0.8 mg)	14675.8	14793.6	–	–	–
Diglycerides	2553.6	2573.2	0.1431	0.00003	0.02
Triglycerides	942.8	976.8	0.0741	0.0012	1.58
Total glycerol	–	–	0.2357	0.00002	0.01

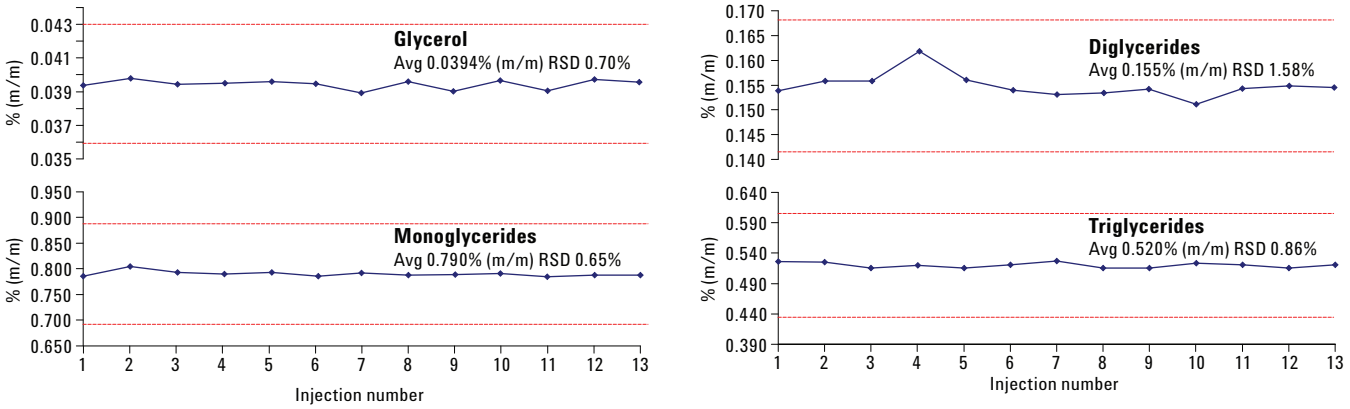

Figure 3. Typical repeatability of 13 successive injections of a spiked biodiesel sample (3S spiked). The red lines represent the maximum variation allowed using the EN-14105 method.

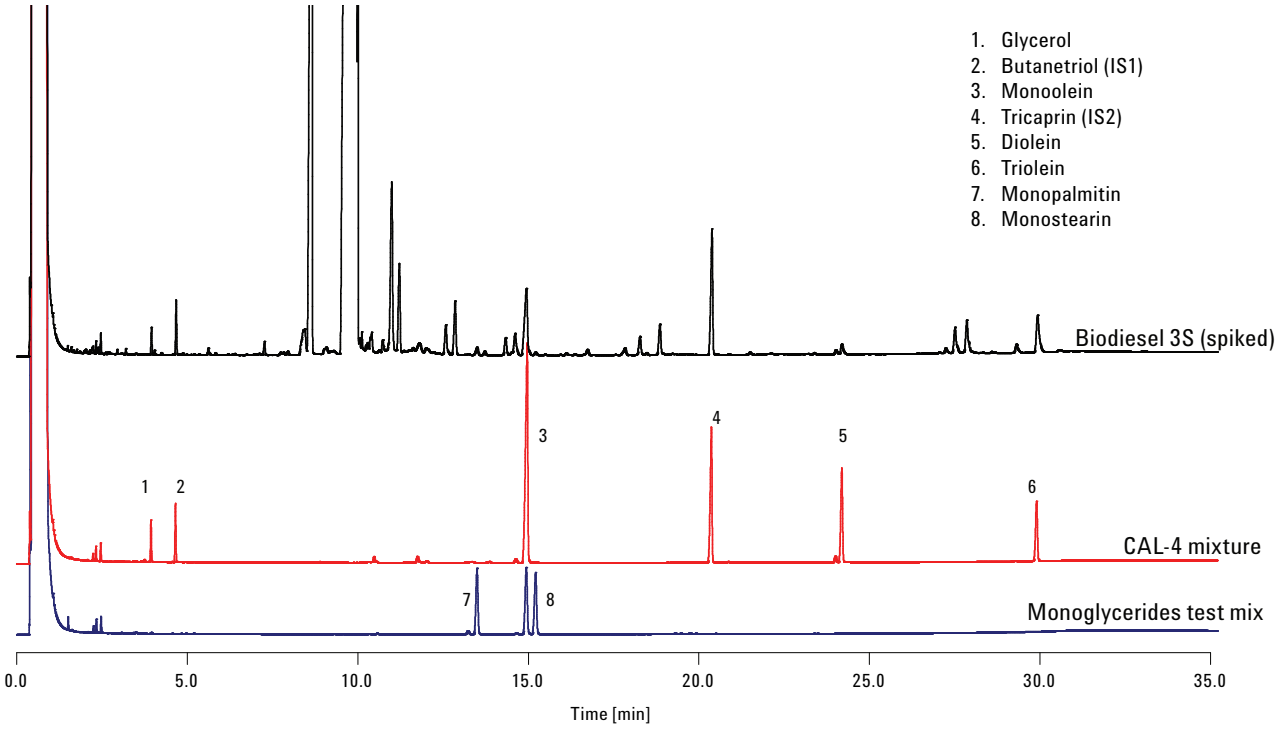

Figure 4. Details of identification of unknown peaks in a biodiesel sample .

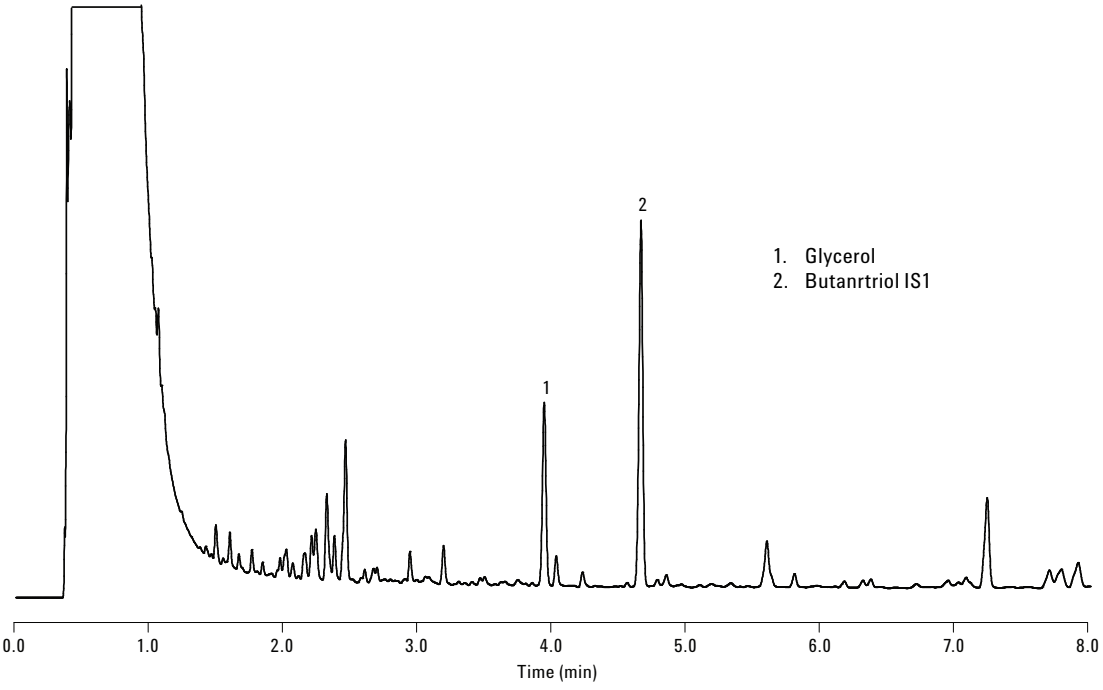

Figure 5. Example chromatogram of B-100 biodiesel (code 3S, with extra glycerol and triglycerides added) with details of the glycerol and internal standard peak.

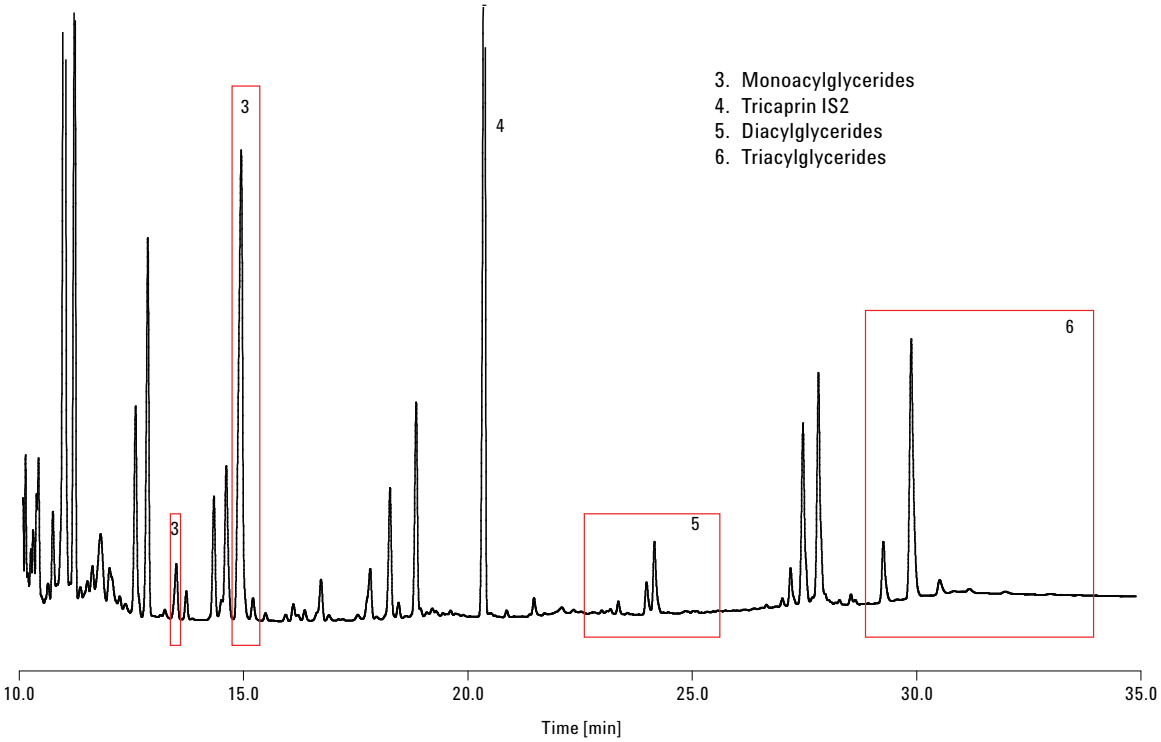

Figure 6. Example chromatogram of B-100 biodiesel (code 3S, with extra glycerol and triglycerides added) with details of the mono-, di-, and triglyceride peak identification and group integration.

The biodiesel sample analyzed was within the EN-14214 specification. However, because of the low levels of both glycerol and triglycerides, the sample was spiked with glycerol and triglycerides so an assessment could be made of the column's separation performance for those components. It is evident in Figure 6 that there is excellent separation of the triglycerides and very low column bleed at 370 °C.

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 glycerol and total glycerol, as well as, mono-, di-, and triglyceride content in biodiesel in accordance with EN-14105 [1].

References

1. EN-14105. Fat and oil derivatives – Fatty Acid Methyl Esters (FAME) – Determination of free and total glycerol and mono-, di-, triglyceride content.
2. Knothe, G. (2006), Analyzing Biodiesel: Standards and other Methods. JAOCS 83, 823–833.
3. EN-14214:2003. Automotive fuels – Fatty Acid Methyl Esters (FAME) for diesel engines – requirements and test methods.

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