APPLICATION NOTE

Gas Chromatography

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Free and Total Glycerol in B100 Biodiesel by Gas Chromatography According to Methods EN 14105 and ASTM® D6584

Introduction

With today's increasing concern for the environment and the depletion of fossil fuel resources comes a greater awareness for alternative fuels, especially for biofuels. One of the more common biofuels is biodiesel, which is a renewable fuel from natural oils such as soybean oil, rapeseed oil or animal fats; it is a substitute for petroleum-diesel fuel.

Biodiesel consists of fatty acid alkyl esters produced by the transesterification reaction of vegetable oils and animal fats (Figure 1). When methanol is used for the transesterification reaction, fatty acid methyl esters (FAME) are formed. In addition to being a renewable fuel, biodiesel is also non-flammable, biodegradable and non-toxic, so it greatly reduces many environmental and transportation risks inherent to petroleum-based fuels.

To ensure high quality, criteria are set for many different properties of biodiesel – these criteria are specified in EN 14214 and ASTM® D6751-07a. The most important criterion for a good-quality biodiesel is the completion of the transesterification reaction. Considering the short introduction to this reaction, it is easy to see why.

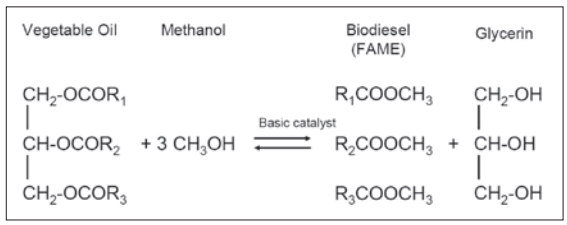

Figure 1. Illustration of the transesterification reaction of triglycerides to fatty acid methyl esters.

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When the triglycerides react with methanol, first, the corresponding diglycerides are formed together with the fatty acid methyl ester (FAME). The reaction continues and the diglycerides lose a second FAME and the monoglycerides are formed. Finally, a third FAME will be lost resulting in the free glycerol. Thus, incomplete reaction will give rise to un-reacted triglycerides from the parent vegetable oil or fat and the intermediates mono- and diglycerides. These are referred to as bound glycerol. Another contaminant found in the final biodiesel is remaining glycerol that has not been removed from the biodiesel during the water washing step. The latter is referred to as free glycerol. The sum of the bound and the free glycerol is referred to as total glycerol.

This paper will present the analysis of free and total glycerol by GC-FID following the methodology of both EN 14105 and ASTM® D6584. Analysis of calibration standards and example biodiesel samples will be presented.

Experimental

Sample preparation is a vital step in this analysis. The glycerol, mono-, di- and triglycerides must be derivitized to reduce their polarity and improve the thermal stability of the molecule. The derivatization technique used is silylation. The derivatization reagent to be used is MSTFA (N-methyl-N-(trimethylsilyl)trifluoroacetamide) – the reaction involves the replacement of the active hydrogen of the hydroxyl-group by a trimethylsilyl-group.

The calibration and internal standards are prepared in pyridine, according to the EN and ASTM® procedure. Prepared calibration solutions are readily available (PerkinElmer Part No. N9331040) – this will simplify method setup, reduce preparation time, minimize the possibility of human error and eliminate the need to prepare dilutions.

The derivatization procedure for both standards and samples is identical. Weigh approximately 100 mg of sample or standard into a vial and record the actual weight. Internal standards are added according to the EN or ASTM® specification, and 100 µL MSTFA (derivatization reagent) is finally added. The samples and standards are allowed to stand for 20 min at room temperature to allow the derivatization reaction to complete. Following derivatization, heptane is added and the vial is capped and shaken. The standards and samples are now ready for analysis.

Equally important to the success of this analysis are the instrumental conditions, supplies and the gas chromatograph (GC) configuration. As is clear from the previous discussion, the components analyzed are not the most favorable for gas chromatography. The high-boiling and thermally-labile compounds require a tightly-controlled injection technique. In GC, the most suitable injection technique to achieve a reproducible and controlled injection is the cool on-column injection. The instrumentation used in this paper is the PerkinElmer® Clarus® GC fitted with the programmable on-column injector.

The analytical column used in this application must meet two major requirements:

1. The internal diameter of the column needs to be sufficiently wide to allow on-column injection
2. The column must withstand high oven temperatures

Table 1. **Detailed Instrument Conditions.**

Sample Introduction	**PSS Injector**
Inlet Program Initial Temperature	60 °C
Hold Time 1	1.00 min
Ramp 1	15 °C/min
Inlet Program Intermediate Temp.	300 °C
Hold Time 2	0.00 min
Ramp 2	30 °C/min
Inlet Program Final Temperature	380 °C
Column Flow	3 mL/min
Injection Volume	1 µL
Gas Chromatograph	**PerkinElmer Clarus GC**
Oven Program Initial Temperature	50 °C
Hold Time 1	1.00 min
Ramp 1	15 °C/min
Oven Program Temperature 2	180 °C
Hold Time 2	0.00 min
Ramp 2	7 °C/min
Oven Program Temperature 3	230 °C
Hold Time 3	0.00 min
Ramp 3	10 °C/min
Oven Program Final Temperature	370 °C
Hold Time 4	5.00 min
Equilibration Time	0.0 min
Column	Elite-Biodiesel M, 14 m x 530 µm x 0.16 µm film
Pre-column	Built-in 2 m Integra-Gap
Carrier Gas	Helium
FID Temperature	380 °C
H_2 flow	45 mL/min
Air flow	450 mL/min
Range	1
Attenuation	-5

You can achieve this 2 ways: a 0.32 mm i.d. fused silica analytical column butt connected to a 0.53 mm i.d. guard-column; or a metal analytical column with 0.53 mm i.d. and integrated guard column. The second option, which is used here, is preferable. The metal column eliminates the physical connection between the analytical column and the guard-column, reducing leaks and breakage. Additionally, the metal capillary column withstands higher oven temperatures, offering a more robust and reliable long-term solution.

Results

The GC analysis of free glycerol, internal standards, mono-, di- and triglycerides identifies each analyte by its retention time. The retention time is determined by the analysis of a known reference standard. Reference standards are also used to generate a calibration curve, which relates FID response to % weight in the samples. The quantification of glycerol, mono-, di- and triglycerides requires a four-level calibration curve for EN 14105 and a five-level calibration curve for ASTM® D6584. An internal-standard calibration is required by both methods.

Table 2. **Summary of Calibration Results for Free and Total Glycerol Following Both EN and ASTM® Methodology.**

	Calibration Summary	
	Linearity (R^2) EN 14110 (4-Point Calibration)	**Linearity (R^2) ASTM® D6584 (5-Point Calibration)**
Glycerol	0.9999	0.9999
Total Monoglycerides	0.9999	0.9984
Total Diglycerides	0.9999	0.9987
Total Triglycerides	0.9985	0.9945

In this application note, calibration curves presented for both EN 14105 and ASTM® D6584 (Table 2) demonstrate excellent linearity, $R^2 > 0.99$ for each analyte. Glycerol is quantified as a single peak, with butanetriol as the internal standard. The monoglycerides are calibrated as a timed group of the 5 monoglycerides, with tricaprin as the internal standard. Total monoglycerides cannot be calculated as a summed time group due to co-elution of the C_{24} ester. The di- and triglycerides are quantified as timed groups, also with tricaprin as the internal standard. The calibration plots are saved in the data processing method of TotalChrom® Chromatography Data Systems (CDS) – a calibration plot is presented in Figure 2.

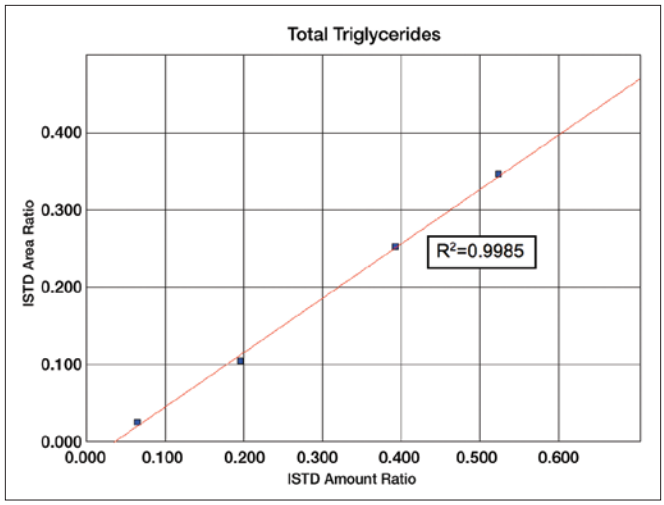

Figure 2. Example calibration plot for total triglycerides following EN 14105.

An example chromatogram of a calibration standard is pictured in Figure 3: glycerol and butanetriol are the first two peaks (callout box 1); following that, monoolein, tricaprin (internal standard), two diglyceride peaks and a single triglyceride peak (callout box 2) elute. The calibration standards only include 1 of the 5 mono-glycerides; EN 14105 requires that a mixture of all 5 monoglycerides be analyzed to determine the retention time of each experimentally, for positive identification. The ASTM® methodology uses relative retention-time data to identify each monoglyceride.

This application note includes the analysis of 2 different biodiesel samples – a washed soy biodiesel and an unwashed used vegetable-oil biodiesel (Figures 4 and 5 respectively). In the washed soy biodiesel sample (Figure 4), the internal standard peaks are clearly visible, along with a cluster of peaks for the FAME content of the sample (9.5-15 min). There are no visible peaks for either free or bound glycerol. This is indicative of a complete reaction and washing. Insufficient washing would demonstrate increased glycerol content. An incomplete transesterification would result in mono- di- and triglycerides detection. In this case, the production facility has a controlled process and a final product which will meet both ASTM® and EN standards.

A biodiesel sample without complete transesterification and washing is pictured in Figure 5. The result is large peaks corresponding to glycerol (callout box 1), monoglycerides (callout box 2), di- and triglycerides (callout box 3). There is also an increase in the presence of matrix peaks (broad peaks around 20 and 22 minutes).

In this case, the production facility needs to modify their procedures and improve the washing step of the process. This will complete the reaction and remove residual glycerol. It would be expected that this sample will have elevated methanol and potassium hydroxide levels, also as a result of incomplete washing.

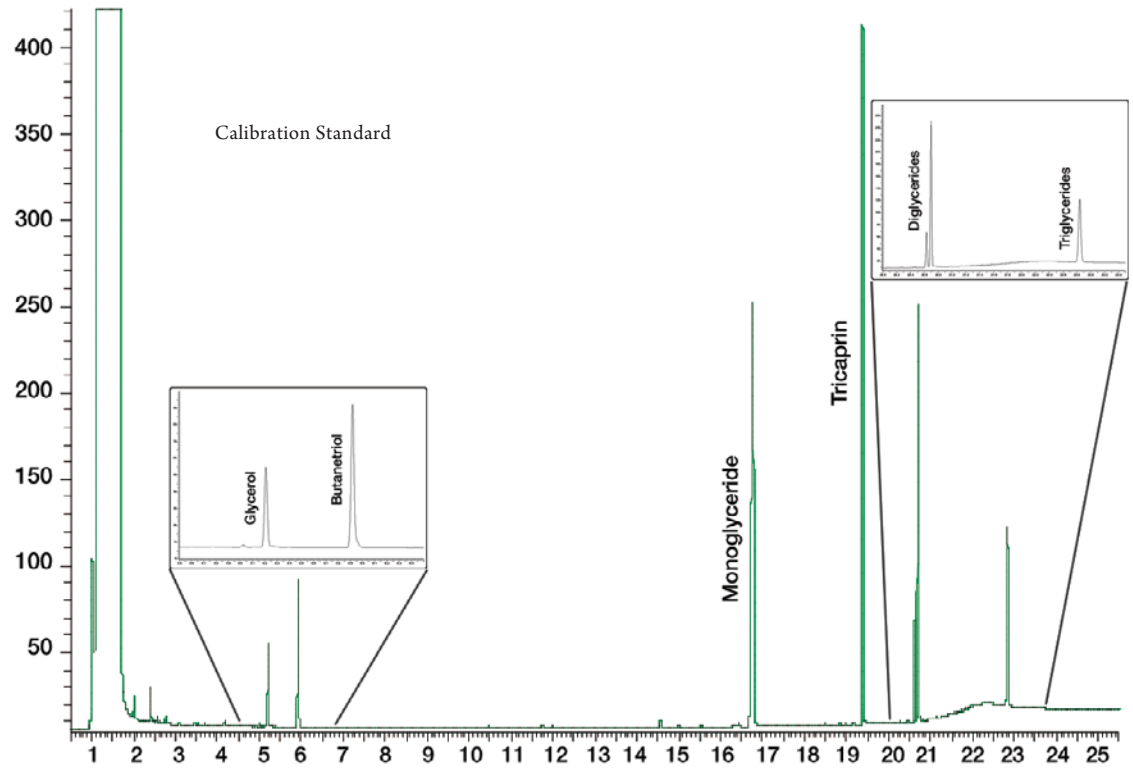

Figure 3. Free and total glycerol calibration standard.

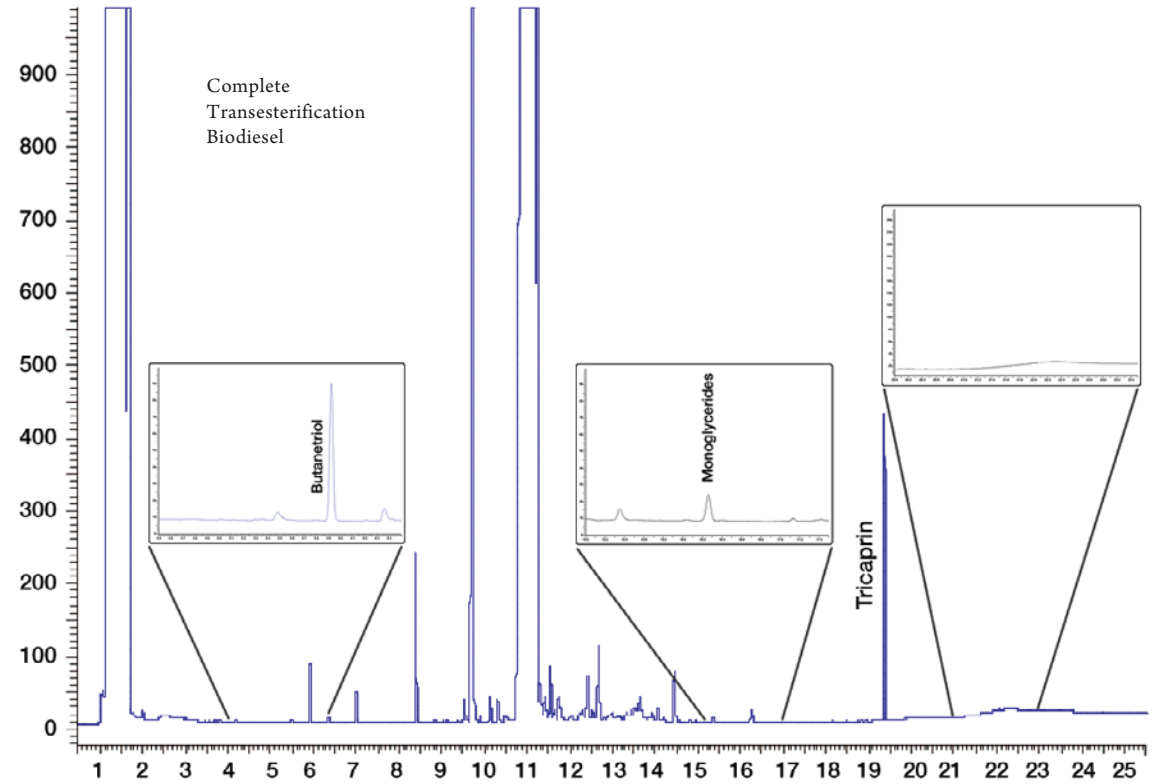

Figure 4. Sample biodiesel in which the transesterification reaction was completed.

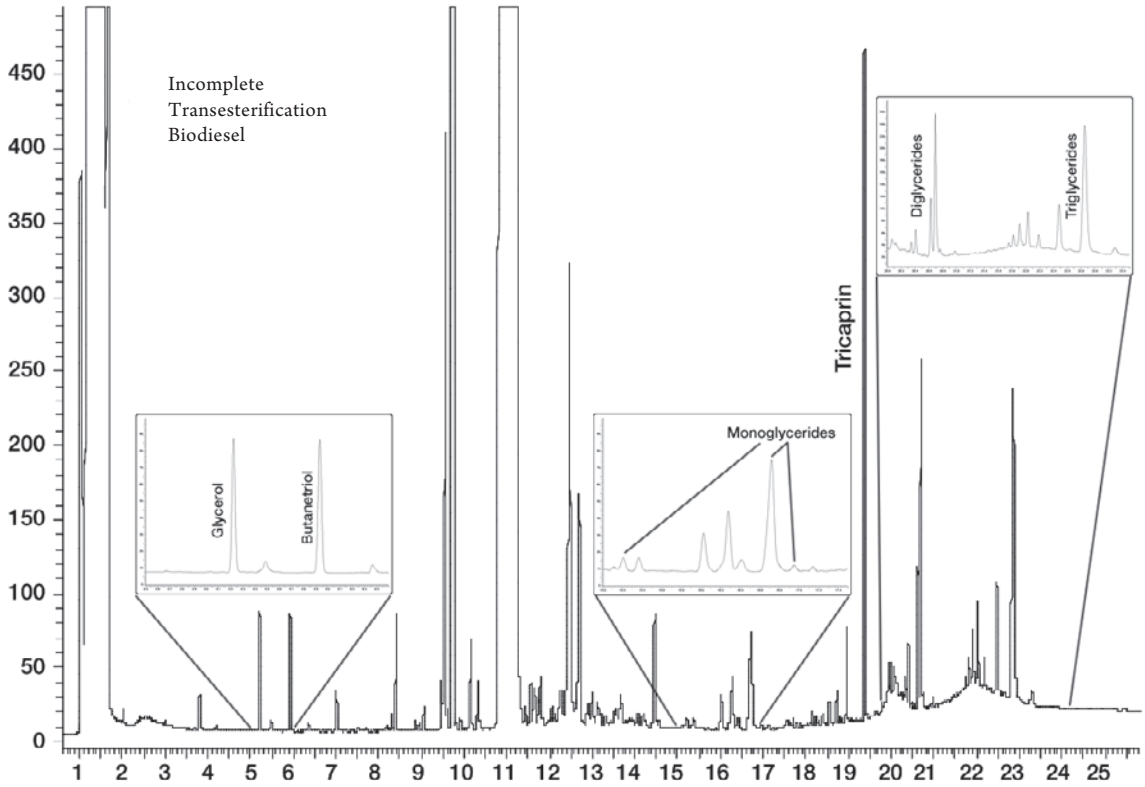

Figure 5. Sample biodiesel in which the transesterification reaction is incomplete.

The final step in the free and bound glycerol analysis is the reporting of % weight results. EN 14105 and ASTM® D6584 present detailed calculations for this determination. The PerkinElmer TotalChrom CDS, used here, will perform the calculations and report the results. An example free-and-bound-glycerol report is pictured in Figure 6.

Component Name	Time [min]	Area [uV*sec]	Amt Mass %	Total Mass %
Glycerol	5.632	5638.38	0.00	0.00
Butanetriol	6.372	145217.36	–	–
Total Monos	17.643	385800.17	0.60	0.16
Tricaprin	19.893	441384.15	–	–
Diglycerides	21.125	43656.13	0.08	0.01
Triglycerides	24.500	515.33	0.01	0.00
				0.17

Figure 6. Example TotalChrom report for free and bound glycerol.

Conclusion

As the distribution of biofuels, particularly biodiesel, expands, the focus on quality becomes more important. Biodiesel is a substitute for petroleum-based diesel fuel – however, the methods to determine fuel quality are different than the traditional methods for the analysis of petroleum fuels. In this paper, the analysis of free and bound glycerol by EN 14105 and ASTM® D6584 was presented. The Clarus GC configured with an on-column injector, flame ionization detector and metal biodiesel capillary column provided the platform for analysis. The system calibration demonstrated linear response for glycerol, mono-, di- and triglycerides. The automation of TotalChrom CDS simplified the calculation and result reporting, delivering a simple report with the percent weight of free and bound glycerol.

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