Analysis of Glycerin and Glycerides in Biodiesel (B100) Using ASTM D6584 and EN14105

Application

HPI/Petrochemicals/Polymers

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Abstract

The analysis of free glycerin (glycerol) and total glycerides (mono-, di-, and triglycerides) in B100 biodiesel was performed according to ASTM method D6584 and CEN method EN14105. Method improvements were demonstrated through the use of a 530-µm id high-temperature fused-silica retention gap coupled to the analytical column. This was made possible with an Agilent Capillary Flow Technology Ultimate Union designed for inert, high-temperature GC oven operation. This configuration on the Agilent 7890A GC System showed calibration and precision performance that exceeded both D6584 and EN14105 specifications. This application provides complete system configuration as well as guidelines for successful analysis of free glycerin and total glycerides in biodiesel.

Introduction

Biodiesel is a motor or heating fuel produced from renewable vegetable oils or animal fats. With the high cost and limited availability of crude oil, renewable fuels like biodiesel are seen as a way to replace, supplement, or extend traditional petroleum fuels. Biodiesel is produced by a process called transesterification. The vegetable oil is reacted with methanol in the presence of a catalyst to produce a mixture of fatty acid methyl esters (FAME) and glycerin. After removal of the glycerin and other contaminants, the remaining FAME mixture is pure biodiesel. Depending on the oil source, a typical biodiesel contains FAME mixtures having both saturated and unsaturated carbon chains from C_8 to C_{24}. Table 1 shows the distribution and relative amounts of FAME found in biodiesel made from common plant oils.[1]

Pure biodiesel is generally not used as a fuel, but instead it is blended with petroleum diesel. Biodiesel is defined by the notation Bxx, where xx indicates the volume percent of FAME content in the liquid. Using this nomenclature, B100 is pure FAME, B50 contains 50 volume % FAME, B5 contains 5 volume % FAME, etc. Common commercial biodiesel blends are B2, B5, and B20.

Before biodiesel can be sold as a fuel or blending stock, it must first meet a defined standard. ASTM standard D6751 and European Committee of Standardization (CEN) standard EN14214 set similar specifications for biodiesel blending and motor fuels.[2,3] In each standard, an important specification is a limit on the amounts of free glycerin and glycerides in biodiesel. Free glycerin is a byproduct of biodiesel production. Mono-glycerides, diglycerides, and triglycerides are partially reacted oils that may be contaminants in the finished biodiesel. High amounts of free glycerin can cause problems due to separation. High amounts of glycerides and glycerin can result in increased engine deposits. Table 2 shows the limits set by each standard.

Table 1. Distribution and Relative Amounts of FAMEs Derived from Vegetable Oils

	Weight Percent FAMEs											
Oil type	C8:0	C10:0	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C20:0 C18:3	C20:1 C22:0	C22:1
Rapeseed					2–5	0.2	1–2	10–15	10–20	5–10	0.9	50–60
Soybean				0.3	7–11	0–1	3–6	22–34	50–60	2–10	5–10	
Palm				1–6	32–47		1–6	40–52	2–11			
Coconut	5–9	4–10	45–52	13–18	7–10		1–4	5–8	1–3			
Palm kernel	2–4	3–7	44–51	14–19	6–9	0–1	1–3	10–18	1–2		1–2	

Table 2. Free and Total Glycerin Specifications for Biodiesel

	EN14214		ASTM D6571	
	Limit (% m/m)	Test method	Limit (% m/m)	Test method
Free glycerin	0.02 max	EN14105	0.020 max	D6584
Monoglycerides	0.80 max	EN14105	NA	D6584
Diglycerides	0.20 max	EN14105	NA	D6584
Triglycerides	0.20 max	EN14105	NA	D6584
Total glycerin	0.25 max	EN14105	0.240 max	D6584

ASTM and CEN have defined several physical and chemical test methods to meet the standard specifications. An important chemical test measures the free glycerin and glyceride content in B100. Two gas chromatographic methods, EN14105 and D6584, were developed to make this measurement.[4,5] Both are nearly identical in sample preparation, instrument configuration, operating conditions, and reporting. Since glycerin and glycerides are polar and high boiling, they must first be derivatized to improve volatility and reduce activity before injection into the GC. A cool-on-column inlet (COC) and high-temperature capillary column are used to make the analysis of these compounds easier. Another important consideration when using these methods is the source of the biodiesel. Both methods were developed for B100 derived from vegetable oils such as rapeseed, soybean, sunflower, and palm. It is known that these methods are not suitable for B100 derived from lauric acid oils, such as coconut and palm kernel oils.

Experimental

Instrument Configuration

Table 3 lists the details of the GC configuration used for this work. A 530-µm id high-temperature retention gap was used between the on-column inlet and the analytical capillary column to improve sample vaporization and provide easy sample injection using a standard tapered needle syringe. An Agilent Capillary Flow Technology Ultimate Union was used to join the retention gap and the analytical column. Table 4 shows the GC operating conditions used for this analysis.

Standard and Sample Preparation

Commercially prepared stock standards were purchased containing glycerin, monoolein, diolein, triolein, butanetriol (internal standard #1), and tricaprin (internal standard #2) at concentrations specified in the ASTM and CEN methods. A list of these standards and other chemical reagents used for this analysis are shown in Table 3.

Five GC calibration standards were prepared by mixing aliquots of the individual stock standards in proportions specified by the ASTM and CEN methods. After mixing, 100 µL of the derivatization agent, N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) was added to each calibration standard. After 20 minutes, 8 mL of reagent grade n-heptane was added to each calibration standard. These final reaction mixtures were directly injected into the gas chromatograph.

Sample preparation followed the procedure in the ASTM and CEN methods. Two samples of B100, from soybean oil and rapeseed oil, were used for this application. Each sample was run two times over four consecutive days with fresh calibration standards prepared and run for each analysis.

Table 3. System Configuration (SP1 7890-0294)

Standard 7890A GC hardware	
G3440A	Agilent 7890A Series GC
Option 122	Cool-on-column inlet with electronic pneumatics control (EPC)
Option 211	Capillary flame ionization detector (FID) with EPC control
G2613A	Agilent 7683 Autoinjector
Columns	
Analytical column	DB-5ht, 15 m x 0.32 mm id x 0.1-µm film (part no. 123-5711)
High-temperature retention gap	Deactivated fused-silica tubing, 1 m x 0.53 mm id (part no.160-2865-5 comes in 5-m lengths)
Union	Capillary Flow Technology Ultimate Union Kit (part no. G3182-61580)
Union ferrules	0.32-mm column Siltite ferrules (part no. 5188-5362) 0.53-mm column Siltite ferrules (part no. 5188-5363)
Data system	
	Agilent Multitechnique ChemStation
Consumables	
5181-1267	10-µL Teflon fixed autoinjector syringe
Standards and reagents*	
44892-U	Glycerin stock standard, 1 mL, 500 µg/mL in pyridine
44893-U	Monoolein stock standard, 3 mL, 5000 µg/mL in pyridine
44894-U	Diolein stock standard, 2 mL, 5000 µg/mL in pyridine
44895-U	Triolein stock standard, 2 mL, 5000 µg/mL in pyridine
44896-U	Butanetriol internal standard #1, 5 mL, 1000 µg/mL in pyridine
44897-U	Tricaprin internal standard #2, 5 mL, 8000 µg/mL in pyridine
394866-10X1ML	MSTFA derivatization grade reagent N-methyl-N-(trimethylsilyl)trifluoroacetamide
H2198	Reagent grade n-heptane

*Available from Sigma-Aldrich, PO Box 14508, St. Louis, MO 63178, USA

Table 4. Instrument Conditions

Cool-on-column inlet	
Mode	Ramped
Initial temperature	oven track, approx 50 °C
Pressure	7.6 psi helium
Injection amount	1 µL
Initial column flow	3.0 mL/min, constant pressure mode
FID temperature	380 °C
Oven temperature program	50 °C for 1 min, 15 °C/min to 180 °C, hold 0 min 7 °C/min to 230, hold 0 min 30 °C/min to 380, hold 10 min

Results and Discussion

After running the standards, Agilent ChemStation was used to calculate linear calibration curves for glycerin, monoolein, diolein, and triolein. The curves for each compound showed excellent linearity and y-intercepts near zero. These curves are shown in Figure 1. The correlation coefficients (r^2) for each compound exceeded the specification of 0.99 set forth in the ASTM and CEN methods.

Figure 2 shows the typical chromatograms obtained for samples of soybean B100 and rapeseed B100. The large peaks observed in each chromatogram are the FAMEs present in the samples. Figure 3 shows the selected regions of the rapeseed chromatogram where glycerin, monoglycerides, diglycerides, and triglycerides elute. Peak identification for each compound is made using the relative retention times published in the ASTM method (Table 5). The retention time of the first internal standard, 1,2,4-butanetriol, was used to identify glycerin. The retention time of the second internal standard, tricaprin, was used to identify the monoglycerides, diglycerides, and triglycerides.

Using the approach detailed in the ASTM and CEN methods, the amount of glycerin in each sample was calculated with the calibration functions derived from the glycerin calibration curve. Likewise, the amount of monoglycerides, diglycerides, and triglycerides was determined from the monoolein, diolein, and triolein calibration functions, respectively. Table 6 list the amounts of glycerin and glycerides found in each sample.

Precision of the analysis was measured using repeatability, which is the difference between two successive analyses of the same sample run on the same day by a single operator on the same instrument. This repeatability measurement was made for each sample over four consecutive days. Table 7 shows the results of the daily precision measurements compared to the specifications from the ASTM D6584 method. These results show excellent single-day precision as determined by repeatability.

ASTM D6584 and EN14105 are not easy methods to run for a number of reasons: the sample preparation is lengthy and difficult; the sample injection

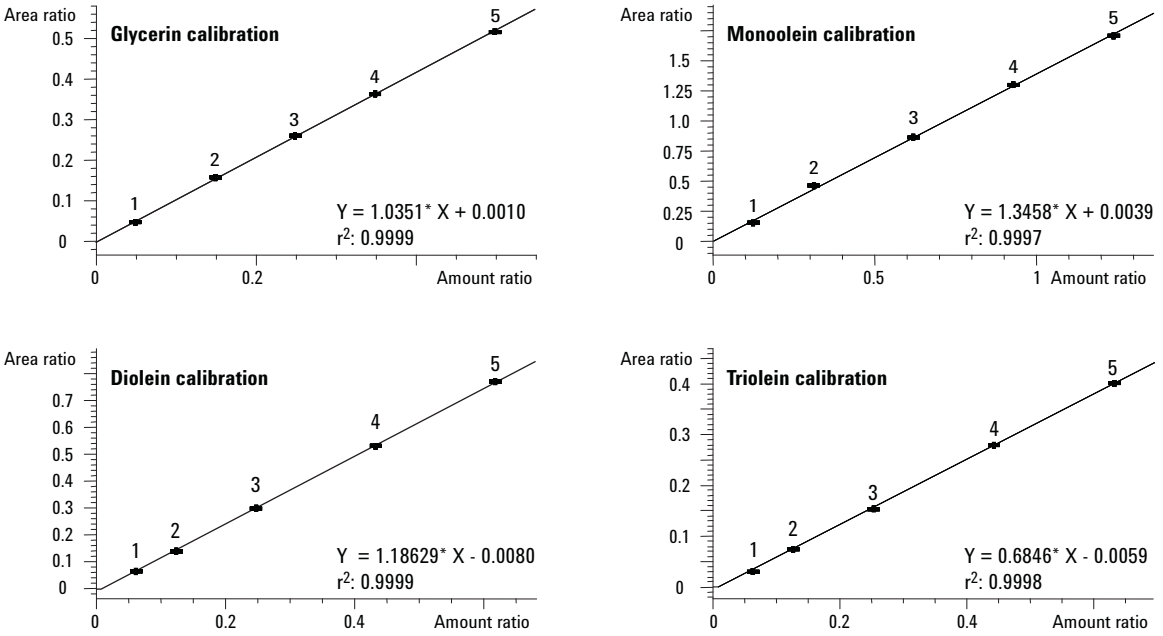

Figure 1. Calibration curves for glycerin, monoolein, diolein, and triolein.

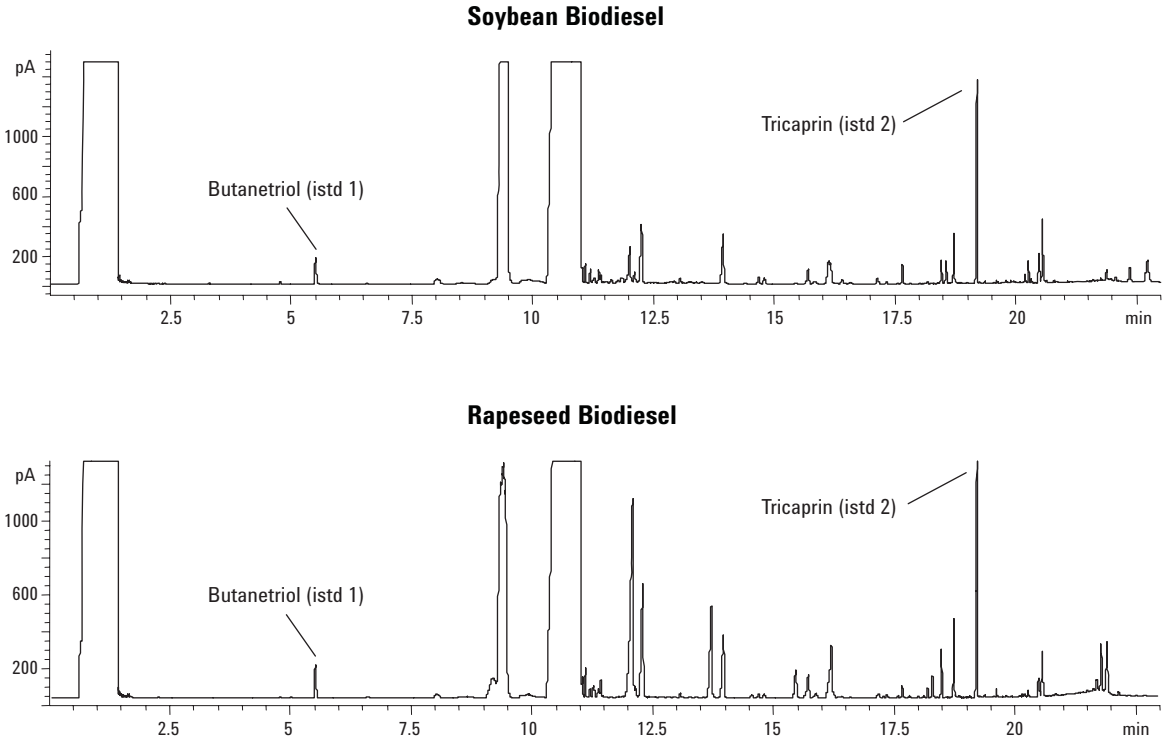

Figure 2. Chromatograms showing typical analysis of free and total glycerins in two B100 biodiesel samples.

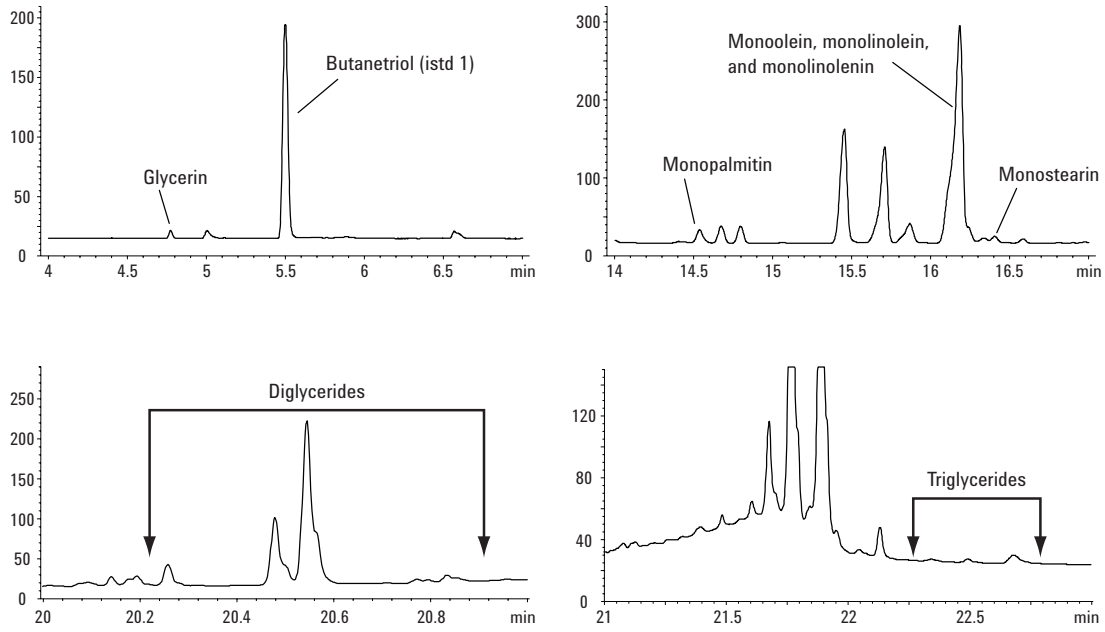

Figure 3. Details of glycerin, monoglycerides, diglycerides, and triglycerides found in a sample of rapeseed B100 biodiesel.

onto a 0.32-mm id column is not easily automated; and calibration can be difficult. However, there are a number of guidelines and procedures that can be followed to obtain good, precise results.

Sample and Standard Preparation

1. Prepare fresh calibration standards every day. Once the standards are prepared they should not be stored for more than several hours.
2. Use commercially prepared stock or final calibration standards packaged in sealed, glass ampoules. If all of the standard solutions are not used in a single day, do not save for later use. Water can accumulate in the solutions and this will inhibit derivatization.
3. Only use derivatization-grade MSTFA. Lesser grades contain solvents that can reduce the effectiveness of the reagent. It is best to purchase MSTFA in small quantities packaged in sealed, glass ampoules. As with the standards, discard any unused MSTFA.
4. Use only clean, dry glassware and pipettes.
5. Only analyze finished product B100. This method should not be used for process samples since high methanol content or water content will inhibit derivatization.
6. Run all samples immediately after preparation. Do not store prepared sample for more than several hours, especially in humid environments.

Table 5. Relative Retention Times Used for Peak Identification

	RRT (int std 1)	RRT (int std 2)
Glycerin	0.85	
1,2,3-Butanetriol (int std 1)	1.00	
Monopalmitin		0.76
Monoolein, monolinolein, monolinolenin, monostearin		0.83 – 0.86
Tricaprin (int std 2)		1.00
Diglycerides		1.05 – 1.09
Triglycerides		1.16 – 1.31

GC Analysis

It is recommended that a retention gap be used between the GC inlet and the column. The retention gap will improve peak shape and sample vaporization, as well as maintain column efficiency. Figure 4 shows the improvement in peak shape for glycerin and 1,2,3-butanetriol when using a 0.53-mm id retention gap. A retention gap will also prolong the column life since it traps any nonvolatile compound contained in the sample. A 0.53-mm id retention gap will also make sample injection easier since it can easily accommodate the standard single tapered syringe needle.

Table 6. Weight Percent of Free and Total Glycerin

	%(m/m) in Soybean B100 Biodiesel			
	Day 1 (avg)*	Day 2 (avg)*	Day 3 (avg)*	Day 4 (avg)*
Free glycerin	0.004	0.004	0.004	0.004
Monoglycerides	0.287	0.280	0.285	0.290
Diglycerides	0.533	0.527	0.533	0.546
Triglycerides	0.387	0.371	0.340	0.304
	%(m/m) in Rapeseed B100 Biodiesel			
	Day 1 (avg)*	Day 2 (avg)*	Day 3 (avg)*	Day 4 (avg)*
Free glycerin	0.002	0.002	0.002	0.002
Monoglycerides	0.365	0.375	0.370	0.371
Diglycerides	0.256	0.262	0.256	0.256
Triglycerides	0.021	0.019	0.018	0.016

*Average of 2 runs per day for each sample.

Table 7. Repeatability Results for Two B100 Biodiesel Samples Over Four Days

	Soybean B100 Biodiesel				
	ASTM D6584 Specification (% m/m)	Observed repeatability (%m/m) Day 1	Day 2	Day 3	Day 4
Glycerin	0.001	0.000	0.000	0.000	0.000
Monoglycerides	0.021	0.005	0.007	0.007	0.000
Diglycerides	0.021	0.008	0.008	0.014	0.000
Triglycerides	0.032	0.008	0.004	0.005	0.000

	Rapeseed B100 Biodiesel				
	ASTM D6584 Specification (% m/m)	Observed repeatability (%m/m) Day 1	Day 2	Day 3	Day 4
Glycerin	0.001	0.000	0.000	0.000	0.000
Monoglycerides	0.021	0.007	0.000	0.006	0.000
Diglycerides	0.021	0.003	0.002	0.000	0.000
Triglycerides	0.032	0.002	0.000	0.001	0.000

One problem with using a retention gap is the high oven temperature (380 °C) required for triglyceride elution. Most fused-silica tubing cannot be used above 350 °C. Also, traditional column unions can leak above that temperature. The Agilent Capillary Flow Technology Ultimate Union combined with special high-temperature fused-silica tubing can solve this problem. The Ultimate Union is made with deactivated stainless steel that can be taken to 400 °C without losing inertness. The high-temperature polyimide coating on the retention gap has extended lifetime up to 380 °C.

Successfully using this Union first requires that the retention gap and column be correctly installed using the metal ferrules designed for the Union. Next, the Union must be completely supported so that no weight is placed on the column connections. A bracket is supplied with the Ultimate Union Kit to support the union fitting to the GC oven wall. Failure to do this will result in a large leak after only a few runs above 350 °C, resulting in column damage. Figure 5 shows a correct installation with the Union supported on its bracket in the GC oven. From this photo it can be seen there is no stress on the column or retention gap. Additionally, to extend the lifetime of this connection, the oven temperature should be kept at 50 °C between analyses. It is also recommended that the Union be checked for leaks before running samples. If a leak is detected, make a new connection to the Union with a new ferrule, and evaluate the column performance before running samples.

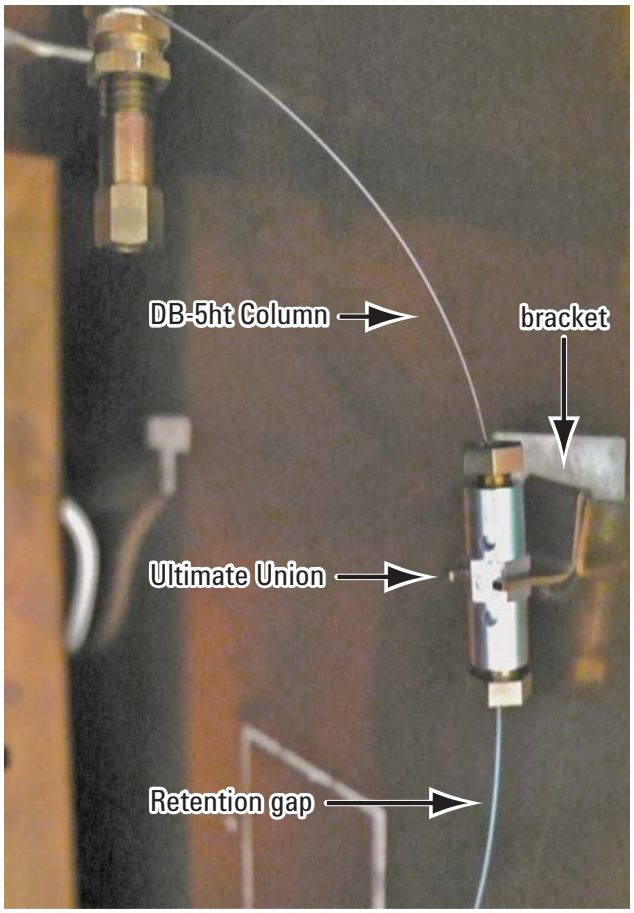

Figure 5. Details of the retention gap and analytical column joined with a Capillary Flow Technology Ultimate Union.

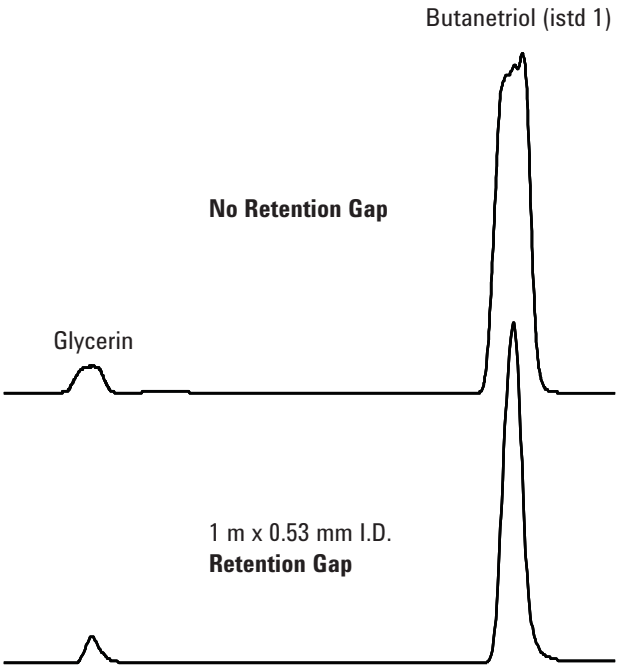

Figure 4. Improved peak shape for glycerin and 1,2,3-butanetriol when using a retention gap and the Capillary Flow Technology Ultimate Union.

Conclusions

The analysis of free and total glycerins can be done using ASTM D6584 or EN14105. Both methods are nearly identical in sample preparation and analysis. This application described the configuration of an Agilent 7890A gas chromatograph for these methods. By combining careful and deliberate sample preparation with a high-temperature retention gap and a Capillary Flow Technology Ultimate Union, this system can obtain results that meet or exceed the methods' calibration and precision specifications.

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