Agilent 7696A Sample Prep WorkBench Automated Sample Preparation for the GC Analysis of Biodiesel Using Method EN14105:2011

Application Note

Fuels

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Abstract

The recently revised European Union method EN14105 describes complex, multistep procedures to manually prepare standards and samples for the GC analysis of glycerol contaminants in B100 biodiesel. The Agilent 7696A Sample Prep WorkBench was successfully used to automate the standard and sample prep of this method while reducing the reagent use and chemical wastes by a factor of 10. Calibration performance of the WorkBench prepared standards exceeded the method requirements. Using a commercial biodiesel sample, the WorkBench was shown to prepare the samples with an extremely high degree of precision that surpassed the method's specifications.

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Introduction

In countries adhering to European Union norms, B100 biodiesel quality is assured by measuring the amount of free and total glycerol and the mono-, di-, and triglycerides contained in the fuel. A gas chromatography (GC) method, EN14105, was developed to separate and quantify these compounds. Since glycerol, mono-, and diglycerides are not volatile, the method outlines a complex procedure to derivatize these compounds and create volatile silanized species prior to GC analysis. In 2011, the European Committee for Standardization (CEN) updated this method to improve GC performance, glyceride quantification, and overall precision [1]. This application note describes using the Agilent 7696A Sample Prep WorkBench to automate the preparation of calibration standards and samples for analysis with the Agilent 7890A Series GC.

The WorkBench is a standalone instrument specifically designed to perform automated sample preparation. It uses two Agilent 7693A injection towers to volumetrically transfer liquids between 2-mL vials. Vials containing various chemical resources, standards, and samples are housed in three 50-positions trays. The sample tray compartment contains a robotic arm, a vortex mixing station, and a sample heating station. For biodiesel analysis, the WorkBench was used to successfully prepare samples for ASTM method D6584, which is similar to the EN14105 method [2]. In that application note, the analysis results from WorkBench prepared samples were identical to results obtained with manually prepared samples.

The Agilent WorkBench Easy SamplePrep (ESP) software was recently updated to provide more efficient use of chemical resources and time. At its core, ESP provides a simple software platform allowing users to quickly build sample preparation methods using drag-and-drop icons representing each WorkBench action. A new mode of ESP operation called **Batch Mode** allows the WorkBench to repeat common actions for all samples before moving on to the next action. For methods where Batch Mode can be used, significant increases in solvent wash and waste capacity can be realized along with faster sample preparation times [3,4].

Experimental

WorkBench Preparation of EN14105 Calibration Standards

The WorkBench was configured with a Blue Line 25 µL gas tight syringe (p/n G4513-80241) in the rear tower and a Blue Line 500 µL gas tight syringe (p/n G4513-60561) in the front tower. The chemical resources used to prepare standards and samples are listed in Table 1. The three reference glycerides used to prepare the Standard Glycerides Solution were purchased as pure compounds from Nu-Chek Prep (www.nu-chekprep.com). Each chemical resource was placed into separate 2-mL high recovery vials (p/n 5183-2030) and sealed using screw caps with PTFE lined septa (p/n 5040-4682).

Table 1. Chemical Resources and Standards used for Method EN14105:2011

Resource	Description	Supplier
Heptane	Capillary GC grade	Sigma Aldrich p/n H9629
Glycerol stock	0.5 mg/mL in pyridine	Sigma Aldrich p/n 44892-U
Butanetriol solution	1 mg/mL in pyridine	p/n 5982-0024
MSTFA	Silanizing reagent	p/n 5190-1407
Std glycerides solution	2.5 mg/mL in THF	Nu-Chek Prep
Monoglycerides RT std	10 mg/mL in pryridine	p/n 5190-1410
Pyridine	Anhydrous grade	Sigma Aldrich p/n 270970

Using the Agilent ESP software, the chemical resources were arranged in the WorkBench and assigned initial properties. This resource layout is described in Table 2 and graphically shown in Figure 1.

Table 2. Agilent WorkBench Chemical Resources used to Prepare Standards and Samples as Shown in Figure 1

Resource name	Resource type	Use type	Capacity (µL)	Vial range
Heptane	Chemical resource	By volume	1,000	81–95
Glycerol stock	Chemical resource	By volume	1,000	61
Butanetriol solution	Chemical resource	By volume	1,000	62
MSTFA	Chemical resource	By volume	1,000	63
Std glycerides solution	Chemical resource	By volume	1,000	64
Monoglycerides RT std	Chemical resource	By volume	1,000	65
Pyridine	Chemical resource	By volume	500	71
Empty vials				51–55

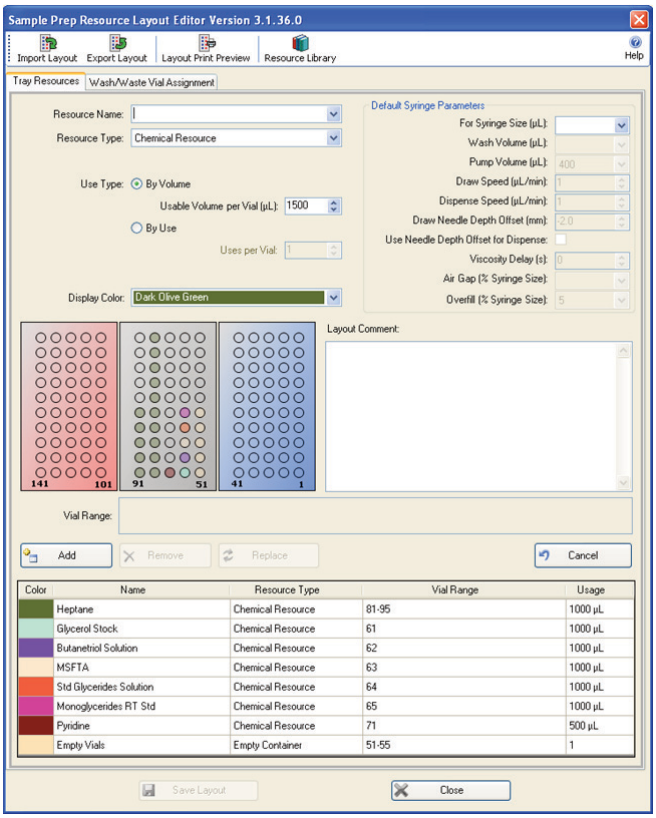

Figure 1. Easy Sample Prep (ESP) software layout for preparing standards and samples using method EN14105.

The EN14105 method requires the preparation of five calibration standards using a linear dilution technique. Four standards contain different amounts of glycerol and the same amount of the internal standard 1,2,3-butanetriol. The fifth calibration standard contains three monoglycerides used to identify these compounds in biodiesel by retention time comparison. The EN14105 method outlines the steps used to prepare approximately 10 mL of each calibration standard. Since the WorkBench uses 2-mL vials, automating the method required a volume reduction by a factor of 10 [2]. Table 3 describes the 37 individual steps used to prepare these five calibration standards. Since this is a linear dilution technique, the ESP Batch Mode was not used for standard preparation (Figure 2). It is important to note that a Needle Depth Offset of 0 was used in combination with the high recovery vials to assure complete mixing of the small volumes needed to prepare these standards. Additionally a 5% Overfill was used when dispensing each resource to eliminate any potential errors causes by bubble formation in the syringe.

WorkBench Preparation of B100 Biodiesel Samples for EN14105

The EN14105 method calls for weighing 100 mg of biodiesel sample into a reaction vial for silation. Since the WorkBench sample prep scale was reduced by a factor of 10, only 10 mg of sample was weighed into 2-mL high recovery vials. Automatic sample weighing cannot be performed using the WorkBench because there is no analytical balance. Since weighing 10 mg of biodiesel can be very challenging, an Eppendorf Reference Adjustable-Volume Pipettor (10–100 µL) was used to transfer the sample. Weighing 10 mg of biodiesel was done by manually pipetting 11.5 µL of biodiesel into tared 2-mL high recovery vials and recording the weight to the nearest 0.01 mg.

Table 3. WorkBench Method used to Prepare Calibration Standards for Method EN14105

Step	WorkBench action	Description	Syringe	Draw speed (µL/min)	Dispense speed (µL/min)	Needle depth offset (mm)	Viscosity delay (sec)	Overfill %
1	Wash	Syringe three times with 5 µL of butanetriol	25 µL	250	1,000		0	
2–6	Add	8 µL butanetriol to empty vials 1, 2, 3, 4, 5	25 µL	250	1,000	0	2	5
7	Wash	Syringe with wash solvent A	25 µL	250	1,000		0	
8	Wash	Syringe with 5 µL of glycerol stock	25 µL	250	1,000		0	
9	Add	1 µL glycerol stock to empty vial 1	25 µL	250	1,000	0	2	5
10	Add	4 µL glycerol stock to empty vial 2	25 µL	250	1,000	0	2	5
11	Add	7 µL glycerol stock to empty vial 3	25 µL	250	1,000	0	2	5
12	Add	10 µL glycerol stock to empty vial 4	25 µL	250	1,000	0	2	5
13	Add	5 µL monoglyceride RT std to empty vial 5	25 µL	250	1,000	0	2	5
14	Add	20 µL std glycerides to empty vial 5	25 µL	250	1,000	0	2	5
15	Add	20 µL of pyridine to empty vial 5	25 µL	250	1,000	0	2	5
16	Wash	Syringe three times with wash solvent A	25 µL	250	1,000		0	
17–21	Add	15 µL of MSTFA to empty vials 1, 2, 3, 4, 5	25 µL	250	1,000	0	2	5
22–26	Mix	Empty vials 1, 2, 3, 4, 5 at 2,500 RPM for 15 sec						
27	Wait	15 minutes						
28–32	Add	800 µL heptane to empty vials 1, 2, 3, 4, 5	500 µL	1,250	5,000	0	2	5
33–37	Mix	Empty vials 1, 2, 3, 4, 5 at 2,500 RPM for 15 sec						

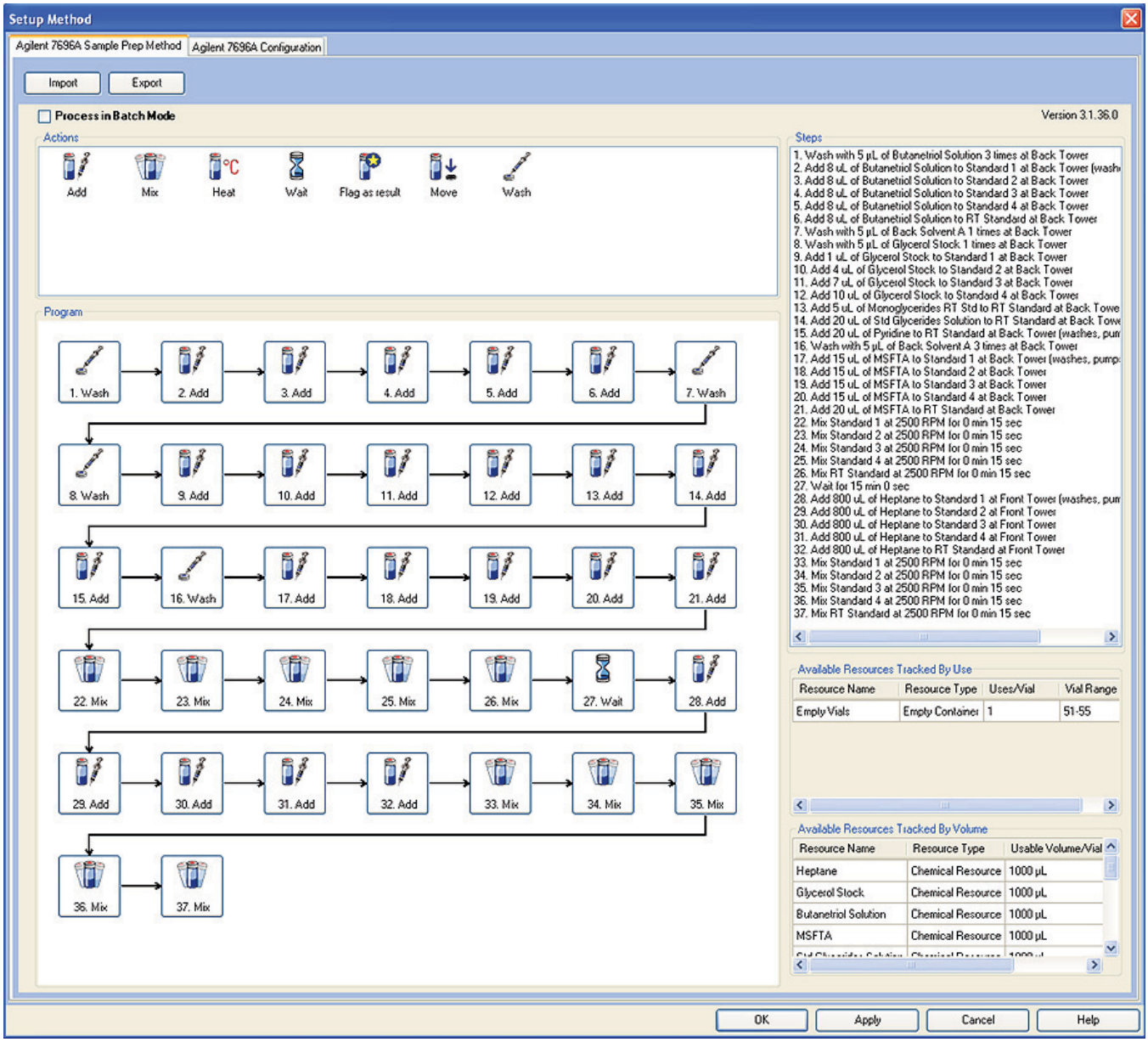

Figure 2. Easy Sample Prep (ESP) software method used to prepare calibration standards for method EN14105.

Sample preparation for the EN14105 method is performed by adding fixed volumes of the butanetriol stock, the standard glycerides stock, pyridine, and MSTFA to the sample to derivatize the non-volatile components. After the 15 minutes, heptane is added to the mix to quench the reaction. Since 2-mL vials were used for the WorkBench, the volumes of each added reagent was reduced by a factor of 10. The individual steps for this sample preparation are listed in Table 4. The ESP software was used to create a Batch Mode method for the sample prep while saving time and resources. This Batch Mode method is shown in Figure 3.

Since both the standards preparation and sample preparation use the same resource layout, the WorkBench can run both methods together using an ESP software Sequence Queue. For this application note, 10 duplicates of a soybean oil derived B100 biodiesel were prepared to evaluate the precision of the WorkBench sample prep.

Table 4. Ten Individual Steps used by the WorkBench to Prepare Biodiesel Samples for Method EN14105

Step	WorkBench action	Description	Syringe	Draw speed (µL/min)	Dispense speed (µL/min)	Needle depth offset (mm)	Viscosity delay (sec)	Overfill %
1	Wash	Syringe three times with 5 µL of butanetriol	25 µL	250	1,000		0	
2	Add	20 µL of pyridine to each sample	25 µL	250	1,000	0	2	5
3	Add	8 µL butanetriol to each sample	25 µL	250	1,000	0	2	5
4	Add	20 µL std glycerides to each sample	25 µL	250	1,000	0	2	5
5	Add	20 µL of MSTFA to each sample	25 µL	250	1,000	0	2	5
6	Mix	Each sample at 2,500 PRPM for 15 sec						
7	Wait	15 minutes						
8	Wash	Syringe one time with 200 µL of wash solvent A	25 µL	250	1,000		0	
9	Add	800 µL heptane to each sample	500 µL	1,250	5,000	0	2	5
10	Mix	Each sample at 2,500 RPM for 15 sec						

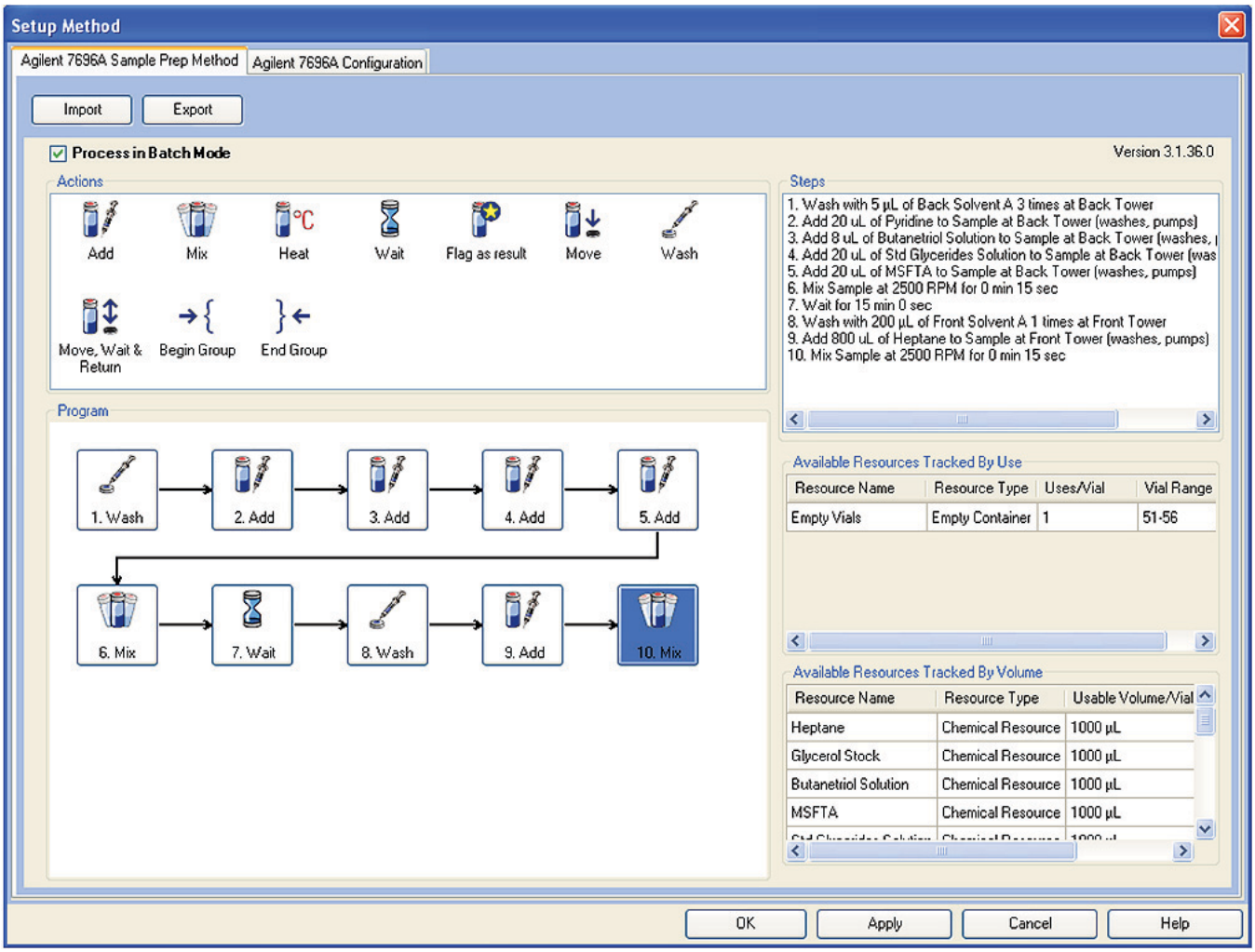

*Figure 3. Easy Sample Prep (ESP) software **Batch Mode** method used to prepare biodiesel samples for EN14105.*

GC Analysis of WorkBench Prepared Standards and Samples

An Agilent 7890A Gas Chromatograph (GC) was configured to comply with the EN14105:2011 requirements. Table 5 lists the instrument configuration and the instrument operating conditions. A single, 1-µL injection of each standard and each sample was made on this system. The Agilent OpenLab CDS Chemstation was used to control the 7890A GC, collect the data, and perform data analysis.

Table 5. Agilent 7890A GC Configuration and Operating Conditions for the Analysis of WorkBench Prepared Standards and Samples using Method EN14105:2011

Instrument configuration	
G3440A	Agilent 7890A Series GC
Option 122	Cool-on-column Inlet with EPC control
Option 211	Capillary FID with EPC control
G4513A	Agilent 7693A ALS
Column	Select Biodiesel for Glycerides
	15 m × 0.32 mm, 0.1 µm film (p/n cp9078)
Data system	Agilent OpenLab CDS Chemstation C.01.03

GC operating conditions	
Cool-on-column inlet	
Initial pressure	Helium at 11.353 psi
Initial temperature	50 °C
Temperature program	Oven track mode
Column flow	Helium at 5 mL/min constant flow
Column temperature	
Initial	50 °C for 1 min
Rate 1	15 °C/min to 180 °C, hold 0 min
Rate 2	7 °C/min to 230 °C, hold 0 min
Rate 3	10 °C/min to 370 °C, hold 10 min
Flame ionization detector	380 °C

Results and Discussion

WorkBench Prepared EN14105 Standards

The retention times of the three monoglycerides and the standard glycerides were determined using the data obtained from the retention time standard. This chromatogram is shown in Figure 4. A glycerol calibration curve was prepared using the data obtained from the four glycerol calibration standards. This curve is shown in Figure 5. The correlation coefficient for this curve was 1.000 which meets the EN14105 method requirement of 0.9.

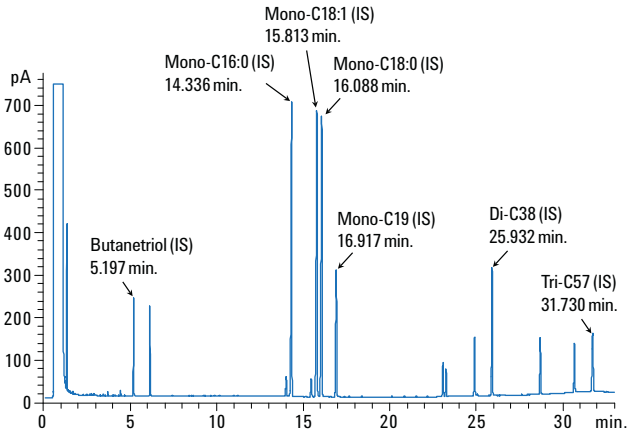

Figure 4. Retention time identification standard prepared using the WorkBench. In addition to the three monoglycerides, the four internal standards (Butanetriol, Mono-C19, Di-C38 and Tri-C57) were also added to this mix.

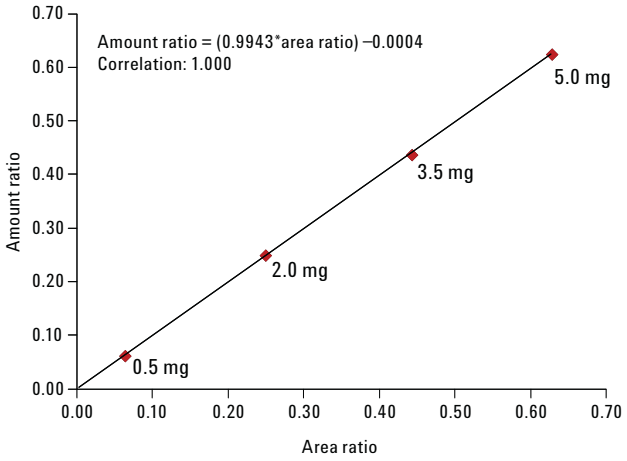

Figure 5. Glycerol calibration curve made using the data from four WorkBench prepared calibration standards. The correlation coefficient exceeds a value of 0.9 as required by the EN14105 method.

WorkBench Prepared B100 Biodiesel Samples

Figure 6 shows a chromatogram of a single sample compared to an overlay of the 10 WorkBench prepared samples. The 10 overlaid chromatograms are nearly identical to the single chromatogram in both retention time and peak response. This result graphically illustrates the WorkBench ability to prepare each sample with precision. Figure 7 shows the four quantification zones in greater detail. Again, these chromatograms are overlays of the 10 WorkBench prepared biodiesel samples and show nearly identical results. In the glycerol and the monoglyceride zones, only the identified peaks are quantified and reported. In the di- and triglyceride zones, any peaks eluting in the respective zone is quantified and reported as a diglyceride or triglyceride.

Before one can determine the final results, a column performance control must be calculated for the analysis. This control is measured by calculating the relative response factors (RRF) of the Di-C38 internal standard versus the Tri-C57 internal standard. The RRF must be lower than 1.8 to be certain of good triglyceride detection. This column performance control was passed for each WorkBench prepared sample as shown in Table 6.

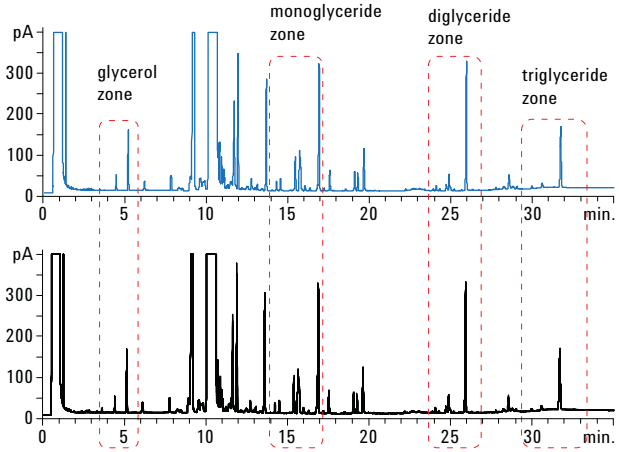

Figure 6. The upper chromatogram is a single run of a B100 sample prepared using the Agilent WorkBench. Each zone for quantification of glycerol and glycerides is outlined in red. The lower chromatogram is an overlay of 10 separate samples prepared using the WorkBench.

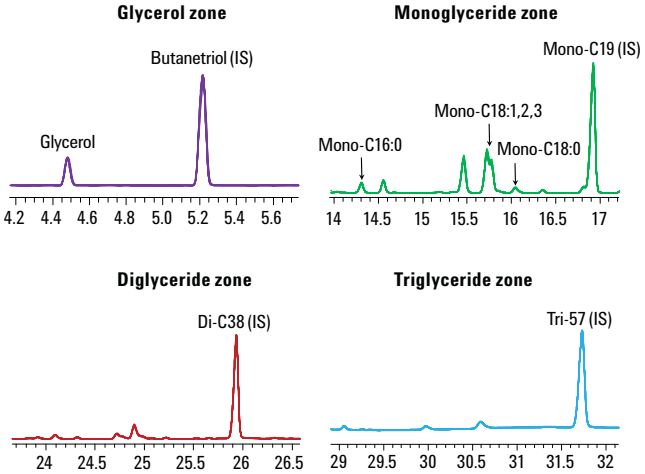

Figure 7. Expanded views of the four quantification zones identified in Figure 5. Note that these chromatograms are overlays of 10 separate samples prepared using the Agilent WorkBench.

Table 6. Column Performance Control Parameters

Sample	A_{DiC38}/M_{DiC38}	A_{TriC57}/M_{TriC57}	RRF
SRM01	24.4	16.5	1.5
SRM02	24.4	16.4	1.5
SRM03	24.4	16.4	1.5
SRM04	24.4	16.4	1.5
SRM05	24.5	16.5	1.5
SRM06	24.6	16.5	1.5
SRM07	24.5	16.0	1.5
SRM08	24.9	16.0	1.6
SRM09	24.9	16.0	1.6
SRM10	25.0	16.2	1.5

As a column performance control, the relative response factor (RRF) for the Di-C38 versus Tri-C57 internal standards must be less than 1.8. All 10 WorkBench prepared biodiesel samples meet this requirement. (A = peak area, M = compound mass)

With the glycerol calibration and column performance control criteria met, the contents of free glycerol, mono-, di-, triglycerides and total glycerol were determined for the 10 WorkBench prepared biodiesel samples. These results are shown in Table 7. The precision for these 10 results was excellent as measured by the low RSDs calculated for each component. However, the EN14105:2011 method does provide a complete statement for both single user and multiple lab precision. For this application note, single user precision can be determined from the results and compared to the method's criteria.

Single user precision is also known as repeatability (r). Repeatability is the difference between two test results obtained by the same operator using the same equipment on identical test material. The EN14105 method provides repeatability statements for each component measured in the sample. To use this statement, the two results with the largest difference, SRM01 and SRM10, were used. The absolute value of the difference for each sample's results was taken and compared to the minimum difference required by the method. As shown in Table 8, samples prepared using the WorkBech comfortably meet the method's repeatability specifications for all quantified components in biodiesel.

Table 7. Results for the Analysis of Ten B100 Biodiesel Prepared using the Agilent WorkBench

		Weight %				
Sample	**Sample weight (mg)**	**Free glycerol**	**Monoglycerides**	**Diglycerides**	**Triglycerides**	**Total glycerol**
SRM01	10.90	0.016	0.39	0.14	0.19	0.156
SRM02	10.40	0.017	0.39	0.14	0.19	0.157
SRM03	10.63	0.017	0.39	0.14	0.19	0.157
SRM04	9.59	0.017	0.39	0.14	0.19	0.157
SRM05	11.12	0.017	0.39	0.14	0.19	0.157
SRM06	9.93	0.017	0.39	0.14	0.19	0.157
SRM07	10.46	0.017	0.39	0.14	0.19	0.157
SRM08	9.66	0.017	0.39	0.14	0.19	0.157
SRM09	9.74	0.017	0.39	0.14	0.19	0.157
SRM10	10.01	0.017	0.39	0.14	0.19	0.157
	Avg	**0.017**	**0.39**	**0.14**	**0.19**	**0.157**
	Std Dev	**0.000**	**0.00**	**0.00**	**0.00**	**0.000**
	RSD	**1.871%**	**0.00%**	**0.00%**	**0.00%**	**0.202%**

Table 8. Analysis Precision as Expressed by Repeatability (r) for two B100 Biodiesel Samples Prepared using the Agilent WorkBench. The Repeatability for Each Component (r calc) Meets the Specification of the EN14105:2011 Method (r spec)

	Weight %				
Sample	**Free glycerol**	**Monoglycerides**	**Diglycerides**	**Triglycerides**	**Total glycerol**
SRM01	0.016	0.39	0.14	0.19	0.156
SRM10	0.017	0.39	0.14	0.19	0.157
r calc	**0.001**	**0.00**	**0.00**	**0.00**	**0.001**
r spec	**0.003**	**0.04**	**0.02**	**0.02**	**0.020**

Conclusion

The Agilent 7696A WorkBench is shown to have successfully performed an automated preparation of standards and samples for the GC analysis of glycerol contaminants in biodiesel according to the revised European Union method EN14105:2011. Since the WorkBench uses 2-mL vials, the scale of the EN14105 preparation was reduced by a factor of 10. This served to lower reagent costs and reduced the generation of waste chemicals when performing this analysis. Calibration standards prepared with the WorkBench met all performance criteria set forth by the method. Ten duplicates of a biodiesel sample were prepared using the WorkBench and the resulting GC analysis showed extremely high precision that exceeded the requirement of the EN14105 method.

References

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