AUTOMATING SAMPLE PREPARATION FOR THE GC ANALYSIS OF BIODIESEL USING METHOD EN14105:2011

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Introduction

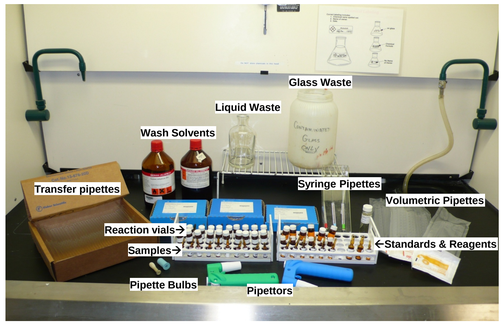
In countries adhering to European Union norms, B100 biodiesel quality is assured by measuring the amount of free and total glycerol and the mono-, di-, and triglycerides contained in the fuel. A gas chromatography (GC) method, EN14105, was developed to separate and quantify these compounds. Since glycerol, mono-, and diglycerides are not volatile, the method outlines a complex procedure to derivatize these compounds and create volatile 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 work describes using the Agilent 7696A WorkBench to automated the preparation of calibration standards and samples for analysis with the Agilent 7890A GC.

1. DIN EN14105:2011-07 "Fat and oil derivatives – Fatty Acid Methyl Esters (FAME) – Determination of free and total glycerol and mono-, di-, and triglyceride contents", European Committee for Standardization, Management Centre: Avenue Marnix 17: B-1000 Brussels.

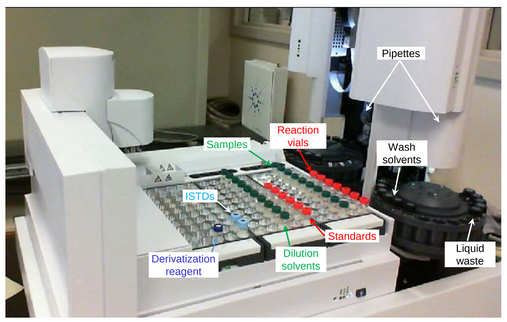
Jet Fuel Contamination with Fatty Acid Methyl Esters (FAMEs)

The Agilent 7696A Sample Prep WorkBench is a standalone instrument specifically designed to perform automated sample preparation. It uses two 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.

A Typical Laboratory Set-Up for Manual Preparation of Standards and Samples for EN14105:2011



A WorkBench Set-Up for Automatic Preparation of Standards and Samples for EN14105:2011



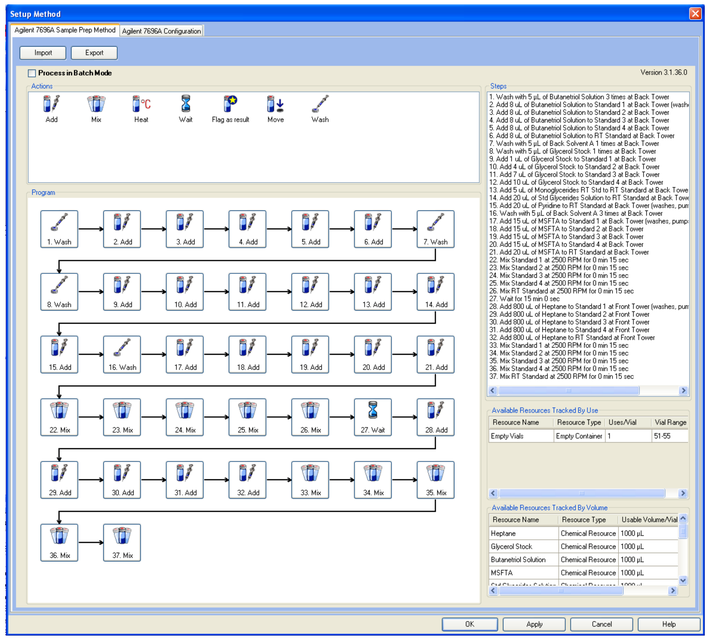
EN14105:2011 Analysis Conditions for the Agilent 7890 GC

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. measured at 50 °C
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	30 °C/min. to 370 °C, hold 10 min.
Flame ionization detector	380 °C

WorkBench Preparation of Calibration Standards

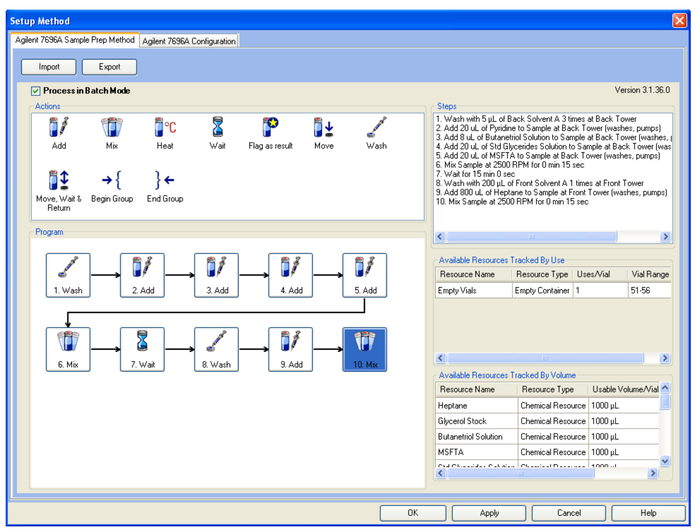
The EN14105 method requires the preparation of 5 calibration standards using a linear dilution technique. Four standards contain different amounts of glycerol and the same amount of the internal standard 1,2,3-butanetriol. The fifth calibration standard contains three monoglycerides used to identify these compounds in biodiesel by retention time comparison. The EN14105 method outlines the steps used to manually 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 ten. Shown below are the individual steps used to prepare the calibration standards using the WorkBEnch software.



WorkBench Preparation of Biodiesel Samples

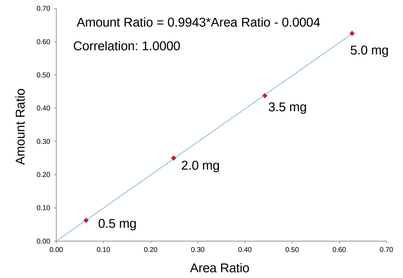
The EN14105 method calls for weighing 100 mg of biodiesel sample into a reaction vial for derivatization. Since the WorkBench sample prep scale was reduced by a factor of ten, only 10 mg of sample was weighed into 2-mL high recovery vials. Automatic sample weighing cannot be performed using the WorkBench because there is no analytical balance. Weighing 10 mg of biodiesel was done by manually pipetting 11.5 uL of biodiesel into tared 2-mL high recovery vials and recording the weight to the nearest 0.01 mg.

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 ten. The individual steps for this sample preparation are shown below.

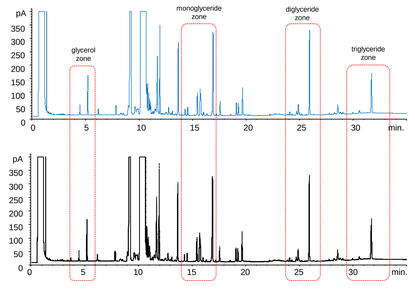


GC Analysis of WorkBench Prepared Biodiesel Samples

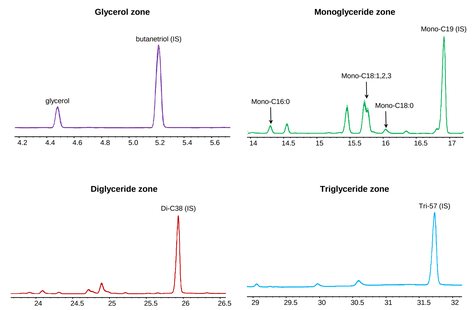
Glycerol calibration curve made using the data from four WorkBench prepared calibration standards. The correlation coefficient exceeds a value of 0.9 as required by the EN14105 method.



The upper chromatogram is a single run of a B100 sample prepared using the Agilent WorkBench. The lower chromatogram is an overlay of 10 separate samples prepared using the WorkBench.



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.



Summary and Conclusion

Sample	Sample amount (mg)	Weight Percent Glycerol	Monoglycerides	Diglycerides	Triglycerides	Total Glycerin
SRM01	10.90	0.016	0.10	0.02	0.02	0.156
SRM02	10.40	0.017	0.10	0.02	0.02	0.157
SRM03	10.63	0.017	0.10	0.02	0.02	0.157
SRM04	9.59	0.017	0.10	0.02	0.02	0.157
SRM05	11.12	0.017	0.10	0.02	0.02	0.157
SRM06	9.93	0.017	0.10	0.02	0.02	0.157
SRM07	10.46	0.017	0.10	0.02	0.02	0.157
SRM08	9.66	0.017	0.10	0.02	0.02	0.157
SRM09	9.74	0.017	0.10	0.02	0.02	0.157
SRM10	10.01	0.017	0.10	0.02	0.02	0.157
	Avg	0.017	0.10	0.02	0.02	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%

Sample	Weight Percent Glycerol	Monoglycerides	Diglycerides	Triglycerides	Total Glycerin
SRM01	0.016	0.10	0.02	0.02	0.156
SRM10	0.017	0.10	0.02	0.02	0.157
r (calc)	**0.001**	**0.00**	**0.00**	**0.00**	**0.001**
r (spec)	**0.001**	**0.01**	**0.01**	**0.01**	**0.014**

The Agilent 7696A WorkBench successfully an automated the preparation of standards and samples for the revised European Union method EN14105:2011. Since the WorkBench uses 2-mL vial, 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.