SHIMADZU

Application Notes

Gas Chromatography

Total Glycerol Contents in Palm Biodiesel by Gas Chromatography

Introduction

The rising price of petroleum-based oil increases the world's interest in alternative fuels. Biodiesel is one of the more popular alternative fuels. The interest in biodiesel arises partly due to the fact that it is a renewable fuel source. Pure biodiesel is basically a group of compounds called Fatty Acid Methyl Esters (FAME), which is made from plant oils or animal fats through a process called transesterification (see Figure A-1 in Appendix 1). In transesterification, the triglycerides from these natural oils are reacted with an excess amount of methanol in the presence of a catalyst.

In Europe and in the United States, there are standards that specify the minimum purity of, or the maximum levels of contaminants in, a pure biodiesel (called B100) before it can be deemed suitable for use as an automotive fuel. EN 14214 and ASTM D6751 are two documents that describe the standard specifications that a pure biodiesel must meet before being used as fuel. These standard documents also specify the analytical methods that can be used to test the purity of, or the impurity levels in, a biodiesel.

Gas chromatography (GC) is one technique that can be used to test three of the specifications of a pure biodiesel, namely: 1) the FAME contents and linolenic acid contents; 2) the total gycerol contents; and 3) the methanol content.

In this Application Note, we describe the gas chromatographic analysis of total glycerol (free glycerol, mono-, di-, and triglycerides) in biodiesel made from palm olein. The analysis was based on EN 14105 standard test method. EN 14105 and ASTM D6584 standard methods were developed for the determination of total glycerol contents in biodiesels produced from sunflower oil, rapeseed oil or soya bean oil. However, in the South East Asian region, one common type of biodiesel is that made from palm olein.

The sample preparation procedure is outlined in Appendix 1, and the reagents used are shown in Table A-2 in Appendix 2.

Identification

The chromatograms of palm biodiesel samples obtained by using a short column (HT5, 10 m x 0.32 mm i.d, 0.10 µm df) and by using a longer column (HT5, 25 m x 0.32 mm i.d, 0.10 µm df) are shown in Figure 1 and Figure 2, respectively.

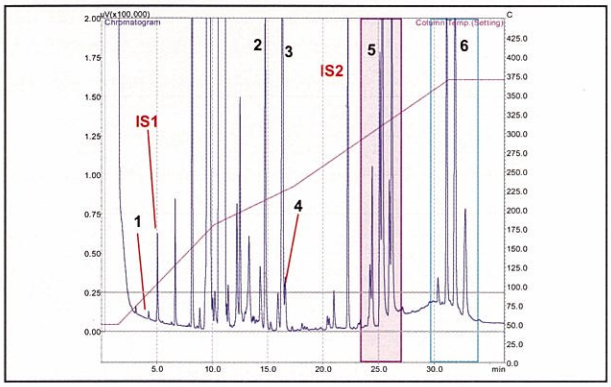

Figure 1. GC/FID chromatographic pattern of a palm olein biodiesel sample. Column: HT5, 10 m, 0.32 mm, 0.10 µm. Method: BS EN 14105:2003. 1: Glycerol, 2: Monopalmitin, 3: Monoolein, 4: Monostearin, 5: Diglycerides, 6: Triglycerides.

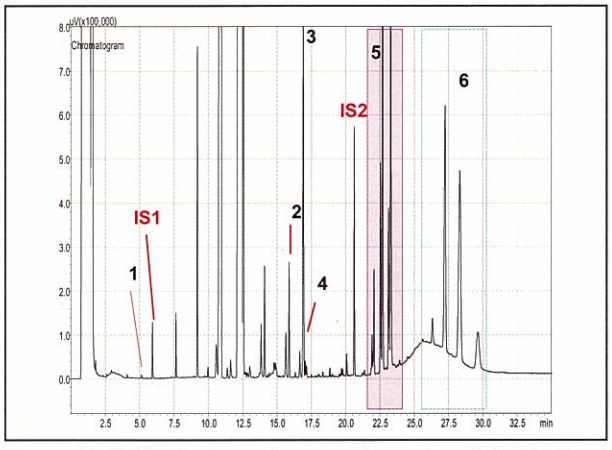

Figure 2. GC/FID chromatogram of a palm olein biodiesel sample. Column: HT5, 25 m, 0.32 mm, 0.10 µm. 1: Glycerol, 2: Monopalmitin, 3: Monoolein, 4: Monostearin, 5: Diglycerides, 6: Triglycerides.

Analytical Conditions

Gas Chromatograph	Shimadzu GC-2010A
Injector	On-Column Injector (OCI-2010) in Simple OCI mode
Injector temp.	60°C (1 min) – 100°C/min – 350°C – 5°C/min – 390 °C (5 min)
Column	HT5 fused silica (SGE), 25 m x 0.32 mm i.d., 0.10 µm df
Column oven temp.	50°C (1 min) – 15°C/min – 170°C – 10°C/min – 230°C – 15°C/min – 380 °C (10 min)
Carrier gas	Helium, at 85 cm/sec (Constant)
Detector / temp.	FID, 390°C

It can be seen here that with the longer column, the triglycerides can still be eluted within a reasonable analysis time (~30 minutes). The main reason we used a longer column was that the part of the column that is most contaminated (i.e. the part connected to the injector) can be removed/cut a few times without significant loss in the resolution in the subsequent analysis.

The monopalmitin, monoolein and monostearin in palm biodiesel were identified by comparing the retention times to the monoglycerides standards. A separate GC/MS analysis[2] confirmed this identification. These monoglycerides were found to be separated satisfactorily from the main FAME components in the biodiesel. The diglycerides in the biodiesel sample were identified to be the peaks eluting about 2 min before diolein peak (a representative chromatogram of one of the standard mixtures is shown in Figure 3). The identity of the diglycerides were confirmed by GC/MS analysis.[2] The triglycerides in the sample were found to be the peaks eluting about 3 min before triolein. The retention times of the analytes are given in Table A-3 in Appendix 2.

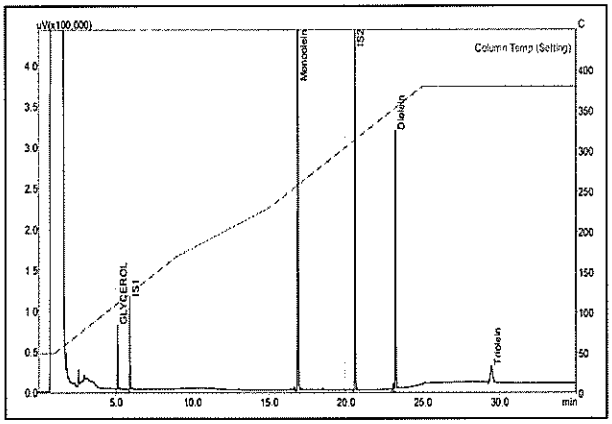

Figure 3. A representative chromatogram of one of the standard mixtures analyzed under the current experimental conditions.

Simple OCI Technique/Mode

For this analysis, we used the Simple OCI injection technique. As is known to many chromatographers, conventionally, in the On-Column Injection (OCI), the liquid sample is deposited directly onto the column. For capillary column, in order to do this, a special OCI syringe whose needle is able to enter the capillary column, must be used. In addition, the column end that is connected to the injector must have an inner diameter (i.d.) of 0.53 mm.

This means that if the analytical column has an i.d. smaller than 0.53 mm, as is the case in this biodiesel analysis, a retention gap or guard column (i.e. a capillary column with no stationary phase) of 0.53 mm i.d. must be connected to the analytical column at the injector end. Connecting two columns with leak-free connection normally requires some skills and some effort.

The Simple OCI technique uses a special deactivated glass insert which allows the liquid sample to be injected onto a column having i.d. between 0.25 mm to 0.53 mm, without having to use a column connector. Better resolution can be expected with smaller i.d. columns. The performance of the Simple OCI technique has been found(1) to be equal to that using the conventional technique.

The glass inserts for the Simple OCI technique can be obtained from Shimadzu (P/N 221-49381-02 for column with 0.32 mm i.d. or larger; P/N 980-00371 for column with 0.25 mm i.d.).

Quantitation

The free glycerol amount was calculated by using internal standard calibration method with 1,2,4-Butanetriol as the internal standard. The total amount of monoglycerides, diglycerides and triglycerides was calculated by using the response factors for monoolein, diolein and triolein, respectively. In turn, the response factors of the monoolein, diolein and triolein were calculated based on internal standard calibration method, using tricaprin as the internal standard. This kind of calculation requires quantitative analysis by using Multi Internal Standard calibration, manual response factor and Group Summation method. GCsolution software was used to perform all the quantitative analysis.

Figure 4 shows the calibration curves for glycerol, monoolein, diolein and triolein. Excellent linear response over the specified range was demonstrated in each case (correlation coefficient, $r > 0.999$). Table 1 shows the summary of the average area response and the average %RSD calculated from four replicate runs of each standard for the concentration range shown.

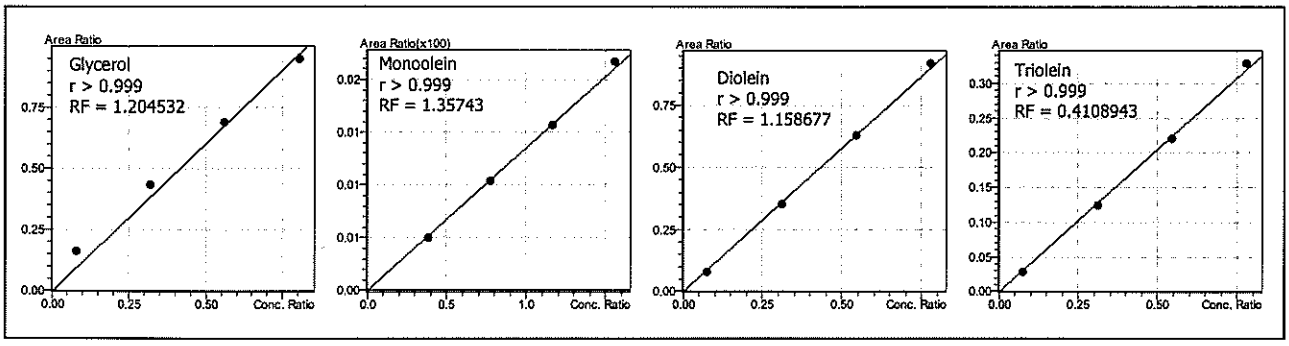

Figure 4. Calibration curves for free glycerol, monoolein, diolein and triolein.

Table 1. Average area responses and Relative Standard Deviations.

Compound Name	Conc. range (mg)	STD 1	STD 2	STD 3	STD 4	Average %RSD
Glycerol	0.005 ~ 0.05	46615	127108	198918	270107	0.88
IS1	-	292926	294888	290555	286580	0.53
Monoolein	0.25 ~ 1	760285	1571130	2345224	3170751	0.44
IS2	-	1546662	1523408	1495964	1466235	0.47
Diolein	0.05 ~ 0.5	122301	530813	940515	1342026	0.59
Triolein	0.05 ~ 0.5	42983	187694	328418	479671	0.90

Standard and Sample Preparation

The calibration standard solutions were prepared in 10 mL gas tight vials by adding appropriate volumes of the individual stock solutions using microsyringes. Table A-1 shows the amounts of the calibration standards and internal standards in each calibration solution (ISTD 1 is 1,2,4-butanediol and ISTD 2 is tricaprin).

Biodiesel sample was prepared by weighing 100 mg of the sample into a 10 mL gas tight vial, then adding the two internal standard compounds.

Prior to analysis, the glycerols in the calibration solution and the sample were derivatized by adding 100 µL of MSTFA (N-Methyl-N-(trimethylsilyl)-trifluoroacetamide) to each of the calibration solution and each sample to be analyzed. The derivatized solutions were analyzed within 24 hours after derivatization due to the limited stability of the derivatization products.

Table A-1. Amount of target analytes in calibration solutions.

Compound Name	Amount of analytes (mg)			
	STD 1	STD 2	STD 3	STD 4
Glycerol	0.005	0.02	0.035	0.05
Monoolein	0.25	0.5	0.75	1
Diolein	0.05	0.2	0.35	0.5
Triolein	0.05	0.2	0.35	0.5
ISTD 1	0.06	0.06	0.06	0.06
ISTD 2	0.64	0.64	0.64	0.64

References

1. Biodiesel Quality Control according to DIN EN 14105 – Determination of free and total glycerol, mono-, di-, triglyceride contents (reference method). Application Note by Shimadzu Europe, SCA_180_016, http://www.shimadzu.de.

2. "Determination of total glycerol content in a palm olein biodiesel by gas chromatography", Novalina Lingga, Analysis Report, CSC 999/GC-07-10, Shimadzu Asia Pacific Pte. Ltd., 2007.

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Appendix 1.

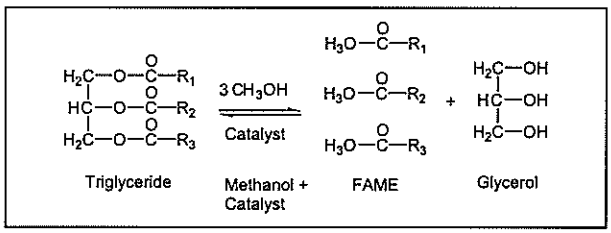

Figure A-1. Transesterification process.

Appendix 2.

Table A-2. Reagents and standards used in this work.

Name	CAS No.	SigmaAldrich Cat. No.	Remarks
Glycerol, anhydrous	56-81-5	49770-250ML	Calibration standard
1-Monooleoyl-rac-glycerol (Monoolein) solution, 5 mg/mL	111-03-5	44893-U	Calibration standard
1,3-Dioleoylglyceride (Diolein) solution, 5 mg/mL	2465-32-9	44894-U	Calibration standard
1,2,3-Trioleoylglyceride (Triolein) solution, 5 mg/mL	122-32-7	44895-U	Calibration standard
MSTFA (N-Methyl-N-(trimethylsilyl)-trifluoroacetamide	24589-78-4	69479-1ML	Derivatization reagent
1,2,4-Butanetriol	3068-00-6	19045-1G	Internal Standard 1
1,2,3-Tridecanoylglycerol (Tricaprin) solution, 8 mg/mL	621-71-6	44897-U	Internal Standard 2
Pyridine, ACS Reagent, 99+%	110-86-1	360570-500ML	Solvent for preparing stock solutions
Heptane (GC grade)	142-82-5	H2198-500ML	Solvent for preparing calibration standard solutions
Monopalmitin (1-Monopalmitoyl-rac-glycerol), 99%	542-44-9	M1640-100MG	Standard for identification
Monostearin (1-Monostearoyl-rac-glycerol), 99%	123-94-4	M2015-100MG	Standard for identification
Monoolein (1-Monooleoyl-rac-glycerol), 99%	111-03-5	M7765-100MG	Standard for identification and an alternative to using the ready-made stock solution
1,2,3-Tridecanoylglycerol (Tricaprin), 99%	621-71-6	T7517-1G	An alternative to using the ready-made stock solution
Triolein, 99%	122-32-7	T7140-1G	An alternative to using the ready-made stock solution

Appendix 3.

Table A-3. Retention times and relative retention times of the glycerols.

Compound	Retention Time	Relative to Internal Standard	Relative Retention Time
Glycerol	5.12	1	0.86
1,2,4-Butanetriol (ISTD 1)	5.92	1	1.00
Monopalmitin	15.878	2	0.77
Monoolein	16.911	2	0.82
Monostearin	17.063	2	0.83
Tricaprin (ISTD 2)	20.623	2	1.00
Diolein	23.263	2	1.13
Diglycerides	21.923 - 23.263	2	1.06 - 1.13
Triolein	29.516	2	1.43
Triglycerides	26.327 - 29.516	2	1.28 - 1.43

Figure A-2. GC-2010AF Gas Chromatography for BioDiesel Analysis.

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