Application Note: 10212

Determination of Total FAME and Linolenic Acid Methyl Ester in Pure Biodiesel (B100) by GC in Compliance with EN 14103

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Key Words

- TRACE GC Ultra
- Biodiesel (B100)
- EN 14103
- Linolenic acid methyl ester
- Total FAME

Introduction

In order for biodiesel to be commercialized as pure biofuel or blending stock for heating and diesel fuels, it must meet a set of requirements defined in ASTM D6751 and EN 14214 standard specifications.[1,2] These specifications indicate the maximum allowable concentrations of contaminants in pure biodiesel (B100) finished product, along with other chemical-physical properties necessary for a safe and satisfactory engine operation.

Gas chromatography (GC) is commonly adopted to characterize pure biodiesel (B100) according to the following standard methods:

- **EN 14103:** Determination of Total FAMEs (fatty acid methyl esters) and Linolenic Methyl Ester (C18:3)[3]
- **EN 14105/ASTM D6584:** Determination of Free and Total Glycerine[4,5,6,7]
- **EN 14110:** Determination of residual Methanol[8,9]

Comprehensive Thermo Scientific GC solutions have been developed in compliance with each of these methods, based on the Thermo Scientific TRACE GC Ultra™ and the versatile TriPlus™ autosampler (Figure 1). This application note relates to the determination of total FAME and linolenic acid methyl ester in biodiesel according to EN 14103.

Figure 1: Thermo Scientific TRACE GC Ultra with TriPlus autosampler

The cetane number of biodiesel depends on the distribution of fatty acids in the original oil. Thus a reliable characterization of FAME is essential for a more accurate calculation of the cetane index. EN 14103 is a standard method for determination of esters and linolenic acid methyl ester and can be applied to biodiesel analysis. EN 14103 requires GC analysis with a split/splitless (SSL) or a programmable temperature vaporizing (PTV) injector and a wax column for a detailed separation of FAMEs. This GC analysis provides verification that the esters content in B100 biodiesel is greater than 96.5% m/m and the linolenic acid methyl ester content is lower than 12% m/m, in accordance with the specifications reported in EN 14214:2003, while also allowing the characterization of FAME composition. Calculation of the percentage of FAME is achieved with internal standard calibration. This method is suitable for FAMEs which contain methyl esters between C14 and C24.

Methods

Instrumentation and Reagents

A Thermo Scientific TRACE GC Ultra equipped with a PTV inlet with backflush option and a flame ionization detector (FID), automated by a TriPlus Autosampler for liquids is used, controlled through Thermo Scientific Chrom-Card data system. The analytical column is a polar Thermo Scientific TRACE™ TR-BIODIESEL(F), 30 m, 0.25 mm ID, 0.25 µm f.t. A 10 mg/mL methyl heptadecanoate solution (C17:0) is used as internal standard.

Sample Preparation

Accurately weigh approximately 250 mg of sample in a 10 mL vial, then add 5 mL of the methyl heptadecanoate internal standard solution using a pipette.

Operation of PTV with Backflush

When FAMEs are analyzed, the heavier fraction present in biodiesel samples (like di- and tri-glycerides) enters the column, getting stacked onto the polar phase. This means that a few nanograms of heavy compounds will accumulate inside the column with every analysis, which increases the risk of compromised chromatographic performance after a number of sequences and dramatically reduces the column lifetime.

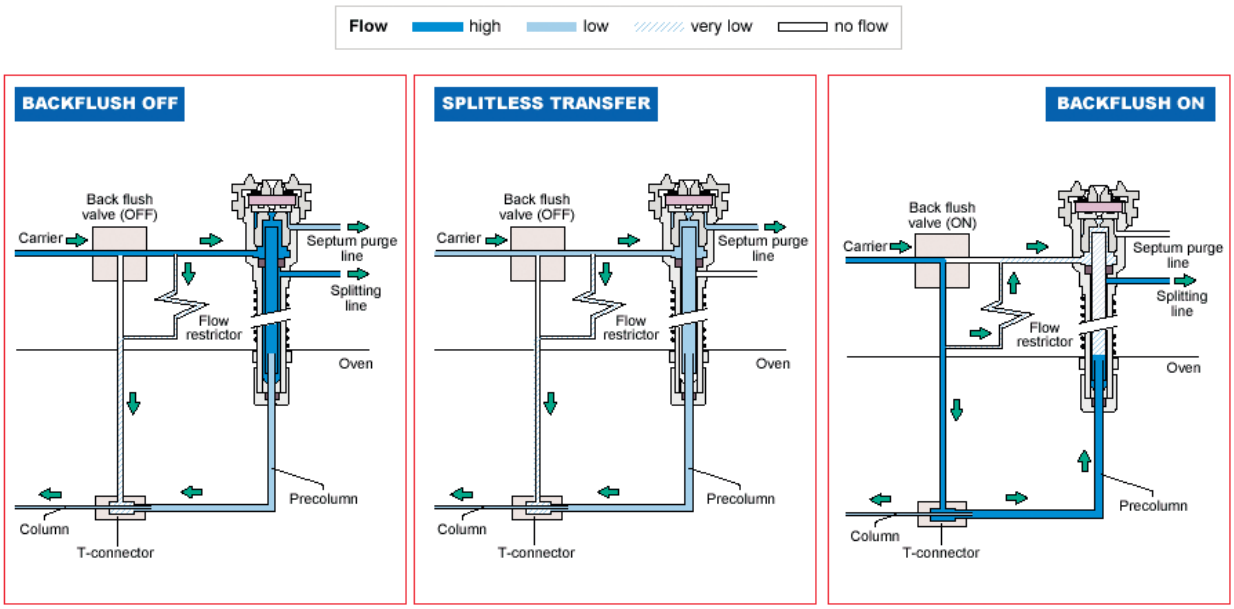

Figure 2: Backflush (reverse flow device): the glycerides fraction is vented out without entering the column

By incorporating the backflush option into the PTV injector, heavy compounds can be vented out of the inlet system, effectively preventing column contamination while still allowing efficient transfer of compounds of interest.

Figure 2 shows how the backflush (or reverse flow device) for the PTV inlet works. The backflush accessory consists of a 3-way solenoid valve (backflush valve) placed in the carrier gas line, a wide-bore pre-column, and a high temperature T connector housed in the GC oven, which connects the pre-column to the analytical column and a calibrated flow restrictor. When the backflush valve is off, the carrier gas flows in its normal direction through the inlet (Figures 2a and 2b). A very small flow, provided by the restrictor, is able to constantly purge the T connector between the pre-column, the analytical column and the backflush inlet line. The pre-column consists of a 2 m x 0.53 mm ID uncoated fused silica tubing, and the purge flow is approximately 5% of the column flow. When the backflush valve is switched on (Figure 2c), the system diverts the gas directly to the T connection at the end of the pre-column, therefore sweeping both the latter and the inlet in the opposite direction, with a so called "reverse flow". In this configuration, the carrier gas is able to "flush" anything still in the pre-column or in the injector directly to the vent through the injector's split line. The small flow provided by the restrictor in the other direction prevents the back-flushed material from flowing through the inlet liner.

Analytical Parameters

Table 1 lists relevant method parameters for the TRACE GC Ultra, and the TriPlus autosampler. Note that the backflush is not activated until 3 minutes have passed, which allows complete transfer of compounds of FAMEs to the analytical column but still ensures that the heavier compounds are vented during the backflush operation.

TRACE GC Ultra and TriPlus AS Autosampler	
PTV Injector	90 °C to 260 °C @ 10 °C/sec, split flow 100 mL/min; Backflush activated after 3 min from injection
Carrier Gas	Helium, 2 mL/min, constant flow mode
FID	280 °C
Oven Program	120 °C (0.5 min) to 220 °C (1 min) @ 30 °C/min, then to 250 °C (5min) @ 10 °C/min
Injection Volume	1 µL

Table 1: Selected instrument parameters

Results and Discussion

Figure 3 shows a chromatogram obtained from a commercial reference rapeseed biodiesel sample analyzed following the conditions reported above, while Figure 4 shows a chromatogram of a real biodiesel produced from unknown source. Table 2 reports the results for both the samples in terms of % m/m of total FAME and of linolenic acid methyl ester. Both the samples tested comply with the specification requirements of EN 14214.

System repeatability was evaluated on the unknown biodiesel, and Table 3 shows that the results well exceed the minimum performance requested by EN 14103. The repeatability was also tested over a sequence of 10 consecutive analyses, getting the results shown in Table 3. The % relative standard deviation (%RSD) of retention times of approximately 0.05% clearly demonstrates the ability of the backflush option to preserve separation and repeatability, even after multiple injections of biodiesel samples.

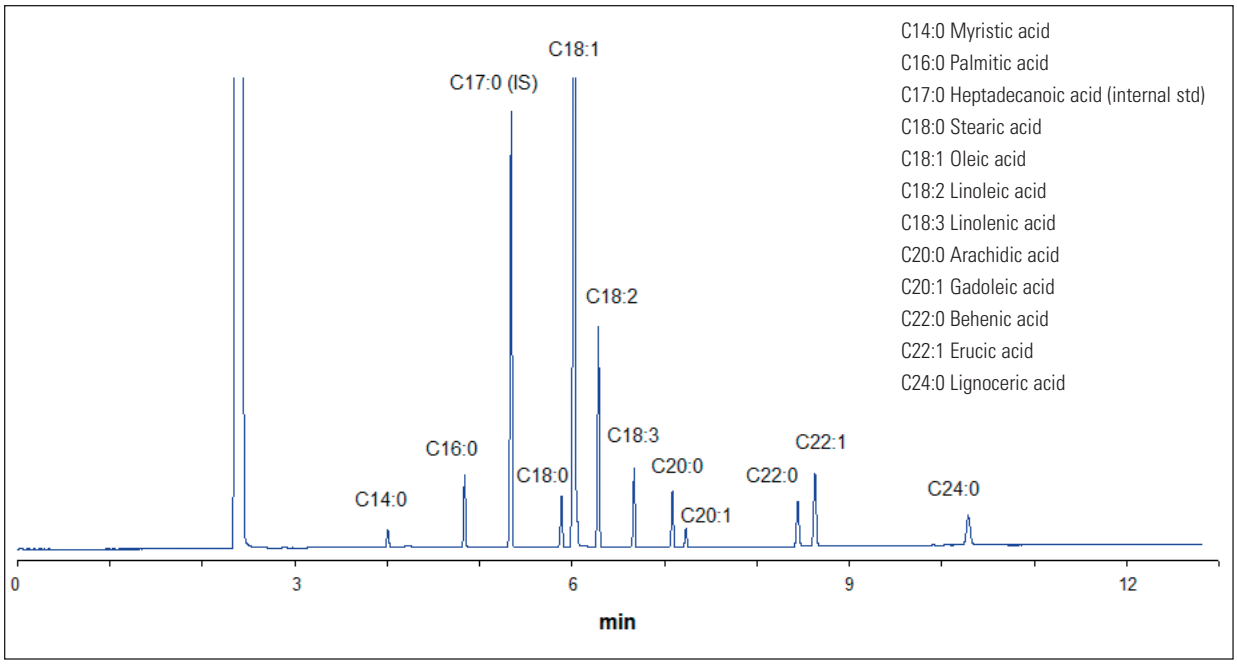

Figure 3: Chromatogram of a reference rapeseed biodiesel

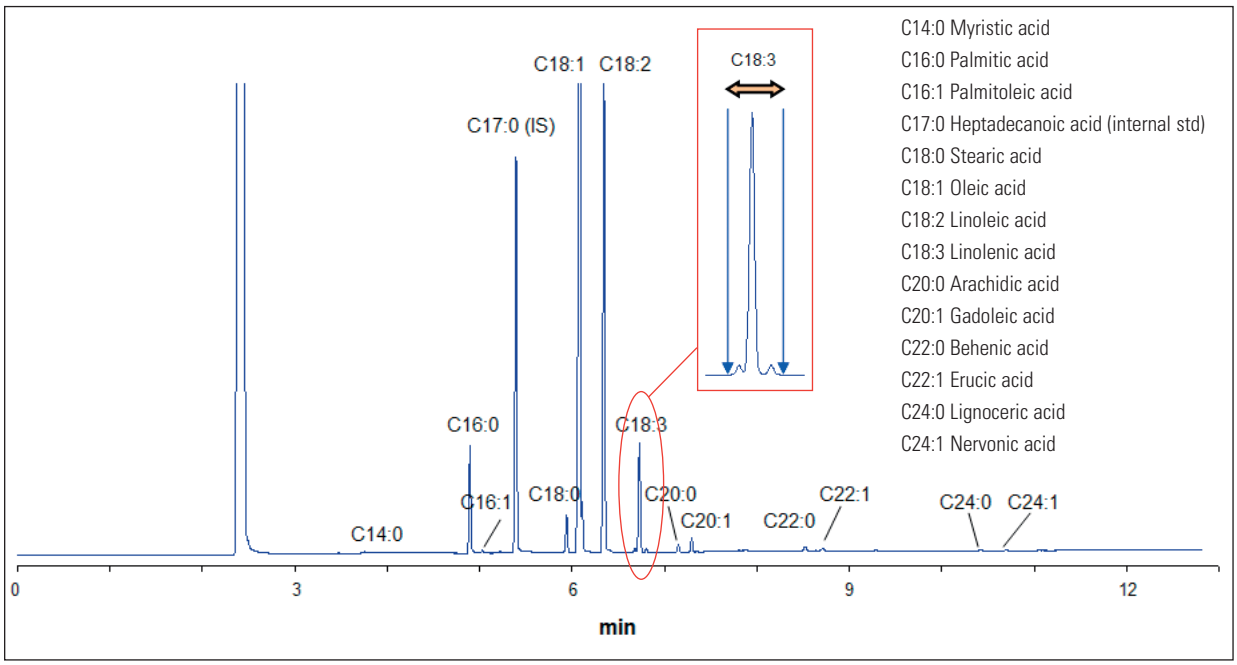

Figure 4: Chromatogram of an unknown biodiesel

	Reference Rapeseed Biodiesel	Unknown Biodiesel	EN 14214 Spec (% m/m)
Total FAME (% m/m)	97.4	96.9	> 96.5
Linolenic Acid (% m/m)	8.3	7.6	< 12

Table 2: Results of 2 biodiesel samples

	Average	Repeatability*	EN 14103 Spec (% m/m)	%RSD (n = 10)
Total FAME (% m/m)	96.9	0.3	< 1.6	0.35
Linolenic Acid (% m/m)	7.6	0.009	< 0.1	0.19

Table 3: Repeatability test conducted on the unknown biodiesel sample

** The absolute difference between 2 independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment with a short time interval (definition reported on EN 14103).*

Conclusion

The determination of total FAME and linolenic acid methyl ester in pure biodiesel (B100) can be achieved in a highly repeatable way using the TRACE GC Ultra equipped with a PTV backflush inlet and FID detector, and automated by the TriPlus liquid autosampler, in full compliance with method EN 14103. The backflush device preserves column performance by venting out the heavier glycerides fraction before it can enter the column, without affecting the determination of total FAME. The described system is also suitable for the determination of methanol in biodiesel by liquid injection.

References

1. ASTM D 6751: Standard Specification for Biodiesel Fuel Blend Stock (B100) for Middle Distillate Fuels.
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3. EN 14103: Fat and oil derivatives – Fatty Acid Methyl Esters (FAME) - Determination of ester and linolenic acid methyl esters content.
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9. Thermo Scientific Application Note #AN10216: Determination of Methanol Content in Pure Biodiesel (B100) by Headspace-GC in Compliance with EN 14110. www.thermo.com.

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