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

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Interest in biodiesel as a clean-burning alternative fuel produced from renewable sources such as vegetable oils has risen considerably over the last few years. It is the only alternative fuel to be legally registered with the US EPA and to have fully completed the health effects testing requirements of the 1990 Clean Air Act Amendments. 'Pure' biodiesel (B100) has been designated as an alternative fuel by the US Department of Energy (DOE) and the US Department of Transportation (DOT).

In order for biodiesel to be commercialised 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 finished product, along with other chemical-physical properties necessary for a safe and satisfactory engine operation. The measurement of biodiesel percentage volume in the final blend can be easily done using chemical information characteristic of and specific to the biodiesel product only. The ideal analytical method for a product such as biodiesel would be able to reliably and inexpensively quantify all contaminants even at trace levels with experimental ease and fast response. Traditionally, gas chromatography (GC) is the most widely used method for the analysis of biodiesel due to its high accuracy in quantifying minor components.[3]

GC is commonly adopted to characterise pure biodiesel according to the following standard methods:

- **EN 14103**: Determination of Total FAME (fatty acid methyl esters) and Linolenic Methyl Ester (C18:3)[4]
- **EN 14105/ASTM D6584**: Determination of Free and Total Glycerine [5,6,7,8]
- **EN 14110**: Determination of residual Methanol [9,10]

The hardware required to accomplish reliably these GC methods must be suitable for a non-discriminative injection of both volatile and heavy compounds and for high temperature operation.

Comprehensive Thermo Scientific GC solutions that comply with each of these standards have been developed, based on the Thermo Scientific TRACE GC Ultra and Triplus Autosampler, with accurate optimisation of the most suitable injection techniques, capillary columns and column connection devices. This article investigates the determination of total FAME and linolenic acid methyl ester in biodiesel according to EN 14103 using these instruments.

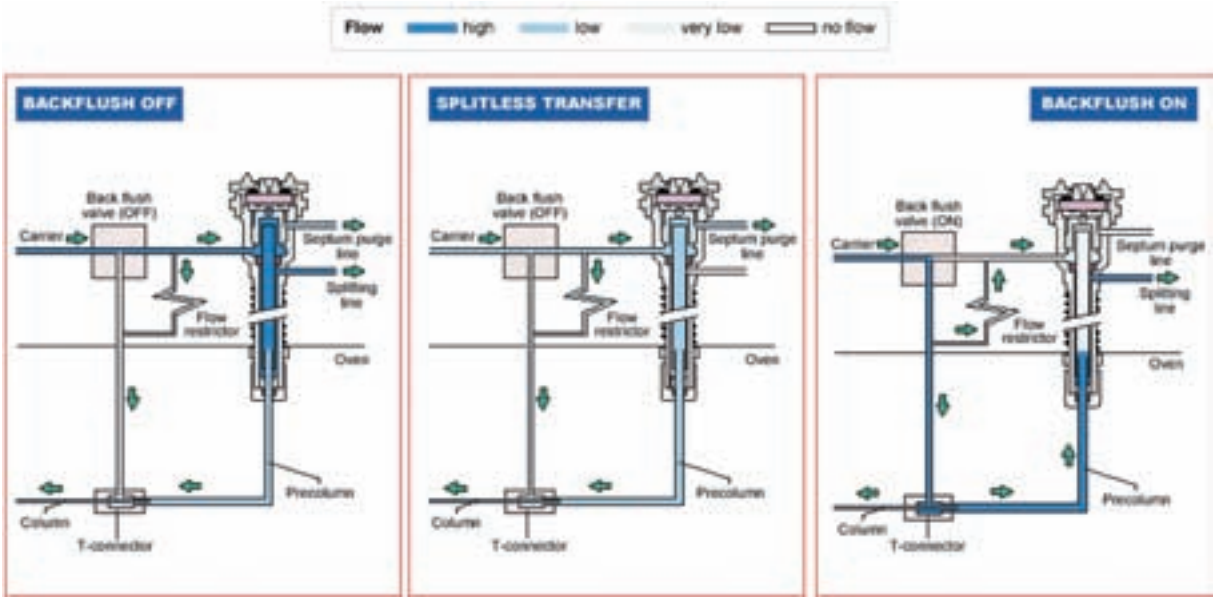
EN 14103

The ester content is an important parameter for determining the presence of substances other than mono-alkyl esters, like unsaponifiable material, which would reveal poor reaction conditions, or contaminants coming from the original oil source. It is therefore a measurement of the feedstock quality and the goodness of the production process.

Besides, the distribution of fatty acids and specifically the degree of unsaturation, is strictly related to other properties like the cetane number and the oxidation stability of the fuel.

The cetane number, a widely used parameter to establish combustion quality for mineral diesel, has been applied as well to alternative diesel fuels as biodiesel and its blends [11]. The cetane number is influenced by the length of fatty acid chain and the number of double bonds. Thus a reliable characterisation of FAME is essential for a more accurate calculation of the cetane index. EN 14103 permits the analyst to assure the B100 product is greater than 96.5% fatty acid methyl esters (m/m) and the linolenic acid content is lower than 12% (m/m), in accordance with the specifications reported in EN 14214:2003, while also allowing the characterisation of FAME composition. Calculation of percentage of FAME is achieved with internal standard calibration, using methyl-eptadecanoate (C17:0) as IS. Since animal fat can contain C17:0, the method is not suitable for this type of feedstock. The analysis is appropriate for FAME compositions between C14:0 and C24:1 and most applicable to C-18 vegetable oils.

EN 14103 requires GC analysis with a split/splitless (SSL) or a programmable temperature vaporising (PTV) injector and a wax column for a detailed separation of FAME.



Figures 1a, 1b and 1c: Backflush (reverse flow device): the glycerides fraction is vented out without entering the column

Backflushing

The main problem with traditional GC is that there is little control over what portion of the sample enters the column in applications including biodiesel analysis. When FAME are analysed, the heavier fraction present in biodiesel samples (like unreacted 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. Backflushing of a capillary column to remove unwanted, less volatile material from the column after the peaks of interest have eluted is a particularly powerful technique offering significant benefits in reducing analysis time and protecting the column.

Methods

Instrumentation and Reagents

A Thermo Scientific TRACE GC Ultra equipped with a PTV inlet with backflush option and a flame ionisation 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

Approximately 250 mg of sample was weighed in a 10 mL vial, then 5 mL of methyl heptadecanoate internal standard solution added using a pipette.

Operation of PTV with Backflush

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 1 shows how the backflush (or reverse flow device) for the PTV inlet works. The backflush accessory consists of a three-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 1a and 1b). 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 1c), 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 three minutes have passed, which allows complete transfer of compounds of *FAME* 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/s, split flow 100 mL/min Transfer Time = 3 min Cleaning: 360°C, split 250mL/min for 20 mins
Carrier Gas	Helium, 2 mL/min, constant flow mode
FID	280°C
Oven Program	120°C (0.5min) to 220°C (1min) @ 30°C/min, then to 250°C (5min) @ 10°C/min
Injection Volume	1uL

Table 1: Selected instrument parameters

Results and Discussion

Figure 2 shows a chromatogram obtained from a commercial reference rapeseed biodiesel sample analysed following the conditions reported above, while Figure 3 shows a chromatogram of a real biodiesel produced from unknown source. Table 2 reports the results for both the samples in terms of percentage 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 according to the definition reported in the method EN 14103 (*), and Table 3 shows that the results well exceed the requested minimum performances. Table 3 reports also repeatability data for a sequence of ten consecutive injections, showing a very good relative standard deviation. Besides, the percentage 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.

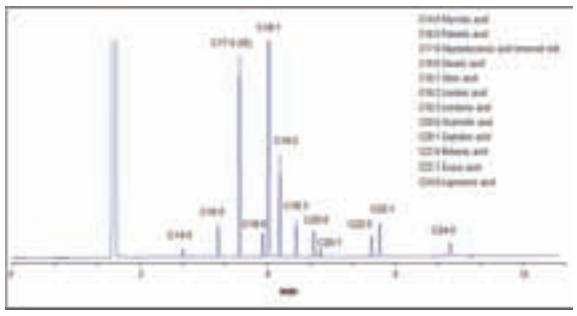


Figure 2: Chromatogram of a reference rapeseed biodiesel

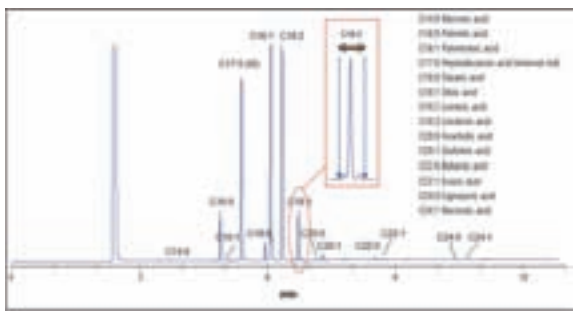


Figure 3: Chromatogram of an unknown biodiesel

	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 two 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 an easier and faster determination of residual methanol in biodiesel by direct liquid injection, exploiting the backflush operation to vent out the heavier fraction and avoid any contamination of the analytical column.

References

1.ASTM D 6751: Standard Specification for Biodiesel Fuel Blend Stock (B100) for Middle Distillate Fuels.

2.EN 14214: Automotive fuels – Fatty Acid Methyl Esters (FAME) for diesel engines – requirements and test methods.

3.Analytical Methods Used in the Production and Fuel Quality Assessment of Biodiesel, G. Knothe, Vol. 44(2): 193-200, Transactions of the American Society of Agricultural Engineers (ASAE) 2001

4.EN 14103: Fat and oil derivatives – Fatty Acid Methyl Esters (FAME) - Determination of ester and linolenic acid methyl esters content.

5.EN 14105: Fat and oil derivatives – Fatty Acid Methyl Esters (FAME) - Determination of free and total glycerol and mono-, di-, tri-glyceride content.

6.ASTM D6584: Standard Test Method for Determination of Free and Total Glycerin in B-100 Biodiesel Methyl Esters By Gas Chromatography.

7.Thermo Scientific Application Note #AN10192: Determination of Free and Total Glycerin in B-100 Biodiesel via Method ASTM D6584. www.thermo.com.

8.Scientific Application Note #AN10215: Determination of Free and Total Glycerin in Pure Biodiesel (B100) by GC in Compliance with EN 14105. www.thermo.com.

9.EN 14110: Fat and oil derivatives – Fatty Acid Methyl Esters (FAME) - Determination of methanol content.

10.Thermo Scientific Application Note #AN10216: Determination of Methanol Content in Pure Biodiesel (B100) by Headspace-GC in Compliance with EN 14110. www.thermo.com.

11.Cetane numbers of branched and straight-chain fatty esters determined in an ignition quality tester, G.Knothe, A. Matheaus, T. Ryan; Fuel, Vol. 82, Issue 8, May 03, p. 971-975