Application note | 001899

Industrial

Biodiesel quality assessment: determination of esters and linolenic acid methyl ester content in biodiesel (B100) by GC-FID, according to EN 14103

Authors

Giulia Riccardino[1], Daniela Cavagnino[1], and Daniel Kutscher[2]

[1]Thermo Fisher Scientific, Milan, IT

[2]Thermo Fisher Scientific, Bremen, DE

Keywords

Fatty acid methyl esters, FAMEs, linolenic acid methyl ester, biodiesel, B100, EN 14103, HeSaver-H_2Safer, gas chromatography, GC, TriPlus RSH SMART, flame ionization detection, FID

Goal

The aim of this study is to demonstrate the suitability of the Thermo Scientific™ TRACE™ 1610 GC series in combination with the Thermo Scientific™ TriPlus™ RSH SMART autosampler for the content assessment of fatty acid methyl esters and linolenic acid methyl ester in pure biodiesel according to the EN 14103 method.

Introduction

Biodiesel is a renewable and biodegradable alternative to mineral fuel, made from vegetable oils through a chemical process of transesterification using alcohol. The process results in two major products: free fatty esters (or biodiesel) and glycerin. When methanol is used for transesterification, fatty acids methyl esters (FAMEs) are obtained. Once separated from glycerin, biodiesel can be blended with petroleum diesel in various concentrations, labeled as BXX, where XX indicates the percentage of biodiesel in the mineral diesel. Today, biodiesel is regularly blended at a level of 5% (B5) and can be used in diesel engines up to 20% (B20) with little or no modifications. Biodiesel quality is critical to ensure a safe and satisfactory engine operation; therefore, during the production process it is important that reaction conversion yield, removal of glycerol, absence of poly unsaturated fatty acids (PUFA), removal of alcohol, and absence of free fatty acids are monitored. The American Society for Testing and Materials (ASTM) and the European Standards (EN) have published analytical methods that are widely adopted to characterize impurities in pure biodiesel (B100) by using gas-chromatography (GC) coupled to flame ionization detection (FID):

- ASTM D6584 for the determination of residual total monoglyceride, total diglyceride, total triglyceride, and free and total glycerin content[1]
- EN 14105 for the determination of residual free and total glycerol and mono-, di-, triglyceride contents[2]
- EN 14110 for the determination of residual methanol[3]
- EN 14103 for the determination of total FAMEs (fatty acid methyl esters) and linolenic acid methyl ester (C18:3) content[4]

This Application Note focuses on the method EN 14103. Refer to AN001897[5] for results on methods EN 14105/ASTM D6584 and to AN001898[6] for results on method EN 14110.

EN 14103

The EN 14103 method provides the instructions for the determination of esters and linolenic acid methyl esters in finished biodiesel by using gas chromatography (GC) coupled to flame ionization detection (FID).

Reliable characterization of FAMEs is essential for accurate calculation of the cetane index of biodiesel. The cetane number is an indicator of diesel fuel quality and represents the readiness of the fuel to auto-ignite when injected into the engine, therefore affecting the engine's starting ability, noise level, and exhaust emissions. The cetane number depends on the distribution of fatty acids in the original oil. In accordance with EN 14214[7] the European Standard indicating the requirements and test methods for biodiesel specification assessment, the total esters content in biodiesel B100 should be greater than 90% m/m and the linolenic acid methyl ester content should be lower than 15% m/m.

Experimental

In this study, a TriPlus RSH SMART Advanced autosampler (Figure 1) was coupled to a TRACE 1610 GC equipped with a Thermo Scientific™ iConnect™ split/splitless injector (iC-SSL) upgraded to work in HeSaver-H_2Safer mode and a Thermo Scientific™ iConnect™ flame ionization detector (iC-FID). A detailed description of the autosampler configuration, including a complete list of suggested consumables is reported in Appendix 1. The Thermo Scientific™ HeSaver-H_2Safer™ carrier gas saving technology[8] offers an innovative and smart solution to dramatically reduce carrier gas consumption, especially during GC operation. When used with hydrogen as a carrier gas, it removes the safety concerns of using hydrogen by limiting the maximum flow supplied to the inlet, even in case of leaks or

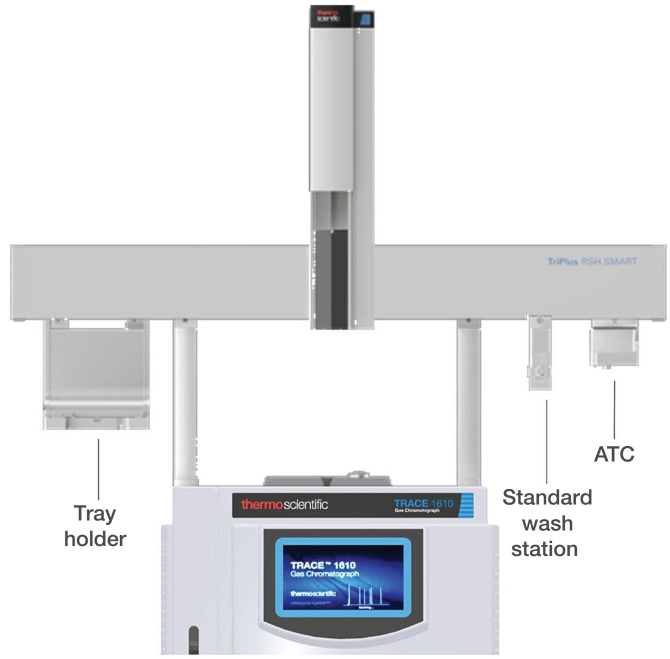

Figure 1. TriPlus RSH SMART autosampler configuration for analysis of methyl esters and linolenic acid methyl ester according to the EN 14103 standard

column breakage. The limited flow makes it impossible to reach a hazardous concentration of hydrogen in the GC oven, removing the need to install a hydrogen sensor.

Chromatographic separation was achieved on a Thermo Scientific™ TRACE™ TR-BioDiesel (F) GC column (30 m × 0.25 mm × 0.25 µm, P/N 26AX142P). This column contains a strongly cross-linked stationary phase and ensures reliable and reproducible performance with low bleed, making it an ideal choice for the analysis of FAMEs according to the EN 14103 standard. In these experiments, hydrogen was used as carrier gas providing a renewable and efficient alternative to helium, while ensuring safe operations with the use of the H_2Safer technology. Detailed experimental conditions set according to the EN 14103 standard are reported in Appendix 2.

For higher throughput, a shorter analysis time can be achieved with the Thermo Scientific™ TRACE™ TR-FAME column (10 m × 0.10 mm × 0.20 µm, P/N 260M096P), which provides efficient chromatographic separation of 37 FAMEs in less than 10 min with resolution of critical pairs (R_s) ≥ 1.0.[9]

Standards

Toluene (purity 99.8+%, Fisher Scientific P/N 10419490) was used as diluent. Nonadecanoic acid methyl ester (100 mg, FAME C19:0, P/N 35055) was purchased from Restek and used as internal standard (ISTD). A standard mix containing a total of 37 individual FAMEs (P/N CRM47885) was purchased from Sigma-Aldrich.

Sample preparation

An aliquot of biodiesel sample (125 mg ± 0.1) and nonadecanoic acid methyl ester (50 mg ± 0.1) were weighed into a 10 mL screw cap vial (P/N 6ASV10-1, caps P/N 6PMSC18-ST2) and diluted with 5 mL of toluene. A sample without the internal standard was also prepared in order to verify the absence of natural occurring nonadecanoic acid methyl ester or other substances co-eluting with the internal standard. The 37-component FAME mix was run to determine the peak retention time of the fatty acid methyl esters.

Sample preparation can be automated thanks to the advanced built-in robotics and sample handling capability of the TriPlus RSH SMART autosampler, which permits the automated addition of the ISTD to the samples using a dedicated prep cycle, and therefore reduces the manual operations to just sample weighing. Automated procedures also allow scaling down sample and ISTD volumes, maintaining highly precise operations.

Data acquisition, processing, and reporting

For the experiments described here, the Thermo Scientific™ Chromeleon™ 7.3 Chromatography Data System (CDS) was used. The instrument control is fully integrated in the CDS, ensuring a streamlined automated workflow for data analysis with minimal user intervention. Moreover, with the ever-evolving compliance requirements for data integrity and data security, Chromeleon CDS provides a secure platform for analytical laboratories to comply with modern regulatory guidelines including FDA 21 CFR Part 11 and European Commission (EU) Annex 11.

Results and discussion

Chromatography

As prescribed in the EN 14103:2020 method, peak integration was carried out for all FAME compounds starting with hexanoic acid methyl ester (C6:0) up to nervonic acid methyl ester (C24:1). No naturally occurring nonadecanoic acid (C19:0) or other co-eluting compounds were found in the analyzed unknown biodiesel sample. Methyl esters and linolenic acid methyl ester content were calculated by applying the formula reported in Equations 1 and 2, respectively, and were within the allowed ranges, with average values of 98.68% (methyl esters) and 7.03% (linolenic acid methyl ester) as reported in Table 1. The analyzed sample showed the typical individual methyl ester composition of a B100 obtained from rapeseed oil, with predominant content of unsaturated fatty acids methyl esters, such as oleic (C18:1, 63%), linoleic (C18:2, 17.5%), linolenic (C18:3, 7.1%), and lower content of saturated fatty acid methyl esters, i.e., palmitic (C16:0) and stearic (C18:0). Chromatograms of the analyzed biodiesel sample spiked with ISTD and the 37 components mix are shown as an example in Figure 2.

Equation (1) $C = (\sum (A_x * R_x) - A_{ei} / A_{ei}) * W_{ei} / W * P * 100$

A_x = peak area of individual methyl ester X in the test sample (from C6:0 to C24:1)

R_x = theoretical flame ionization correction factor (TCF) for FAMEs relative to the ISTD. This value is reported in the EN 14103:2020 standard

A_{ei} = peak area corresponding to ISTD

P = purity of ISTD

W_{ei} = weight, in mg, of the ISTD

W = weight, in mg, of the test sample

Equation (2) $C = (\sum (A_l * R_l) - A_{ei} / A_{ei}) * W_{ei} / W * P * 100$

A_l = peak area of linolenic methyl ester (C18:3) in the test sample

R_x = theoretical flame ionization correction factor (TCF) for linolenic methyl ester relative to the ISTD. This value is reported in the EN 14103:2020 standard.

A_{ei} = peak area corresponding to ISTD

P = purity of ISTD

W_{ei} = weight, in mg, of the ISTD

W = weight, in mg, of the test sample

Repeatability

Methyl esters and linolenic acid methyl esters repeatability were assessed by running a total of n=19 samples. The official method indicates to assess the repeatability by calculating the difference between two test results that does exceed the r value. Whereas the r value for the methyl esters is specified in the method (1.65), the r value for linolenic acid methyl esters needs to be calculated by applying the formula in Equation 3. The difference between two test results obtained in the normal and correct operation of the same method over a total of n=19 injections did not exceed the r values specified in the method, as reported in Figure 3.

Equation (3) $r = 0.0092 * (X + 3.9180)$

X = average of two test results being compared

Carry-over

Carry-over was assessed by running a solvent (toluene) blank before and after a sequence of samples (n=40). The overlaid chromatograms of a solvent blank acquired at the beginning and end of the sequence demonstrated the absence of carry-over from the heavier fraction possibly present in biodiesel samples (Figure 4).

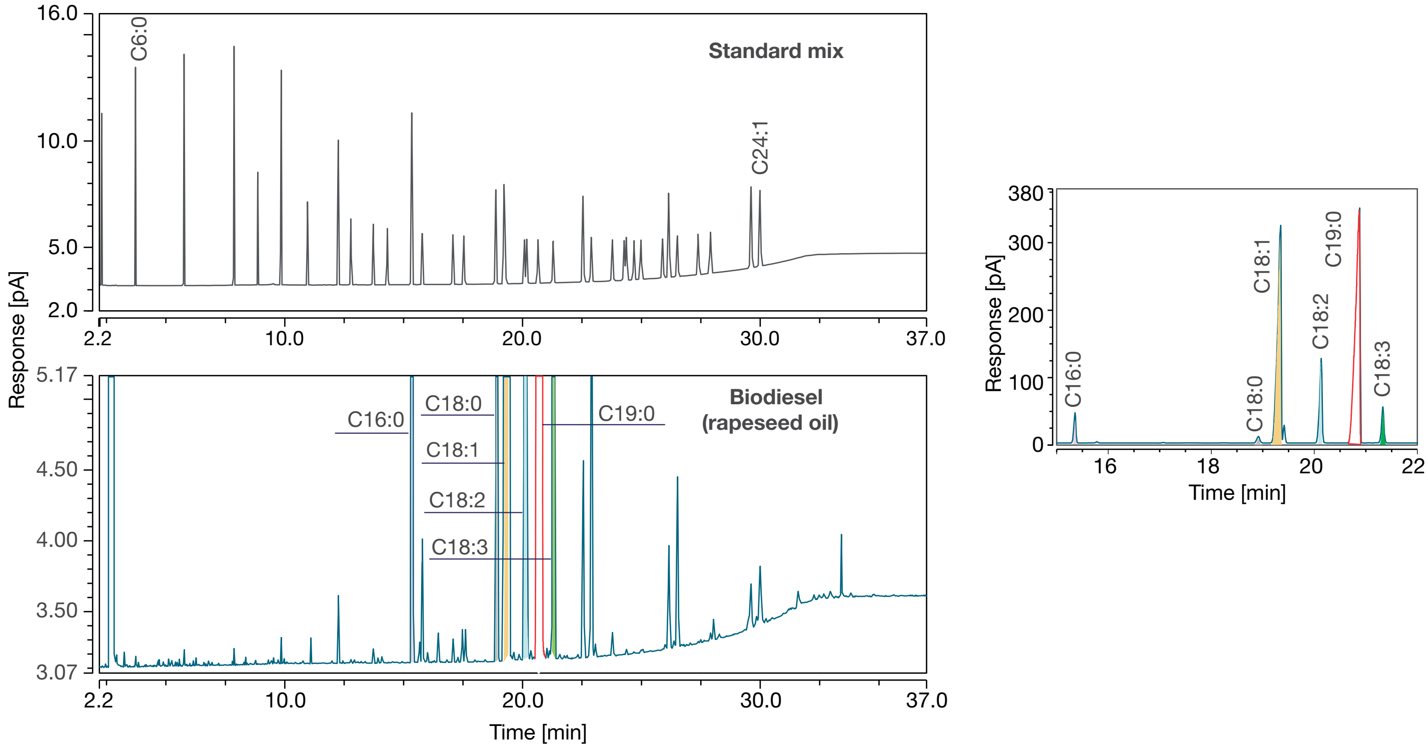

Figure 2. Typical chromatograms for 37 components FAME mix and biodiesel sample from rapeseed oil. The peak integration area specified in the method is from C6:0 to C24:1. The spiked ISTD (C19:0) in the biodiesel sample is marked in red. The inset shows a zoomed-out view of the individual FAMEs typically found in B100 obtained from rapeseed oil.

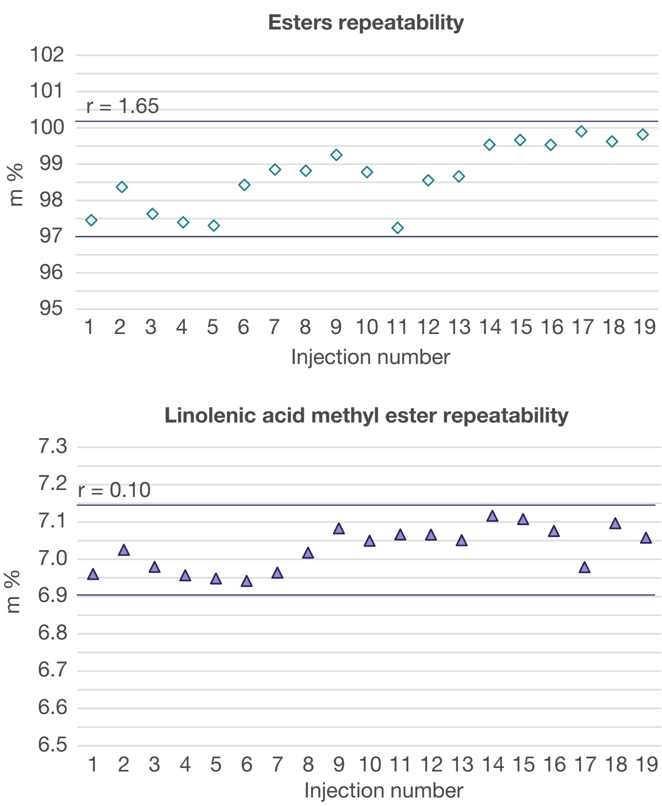

Figure 3. Repeatability evaluated according to the EN 14103 standard by calculating the difference among n=19 replicates of the same sample

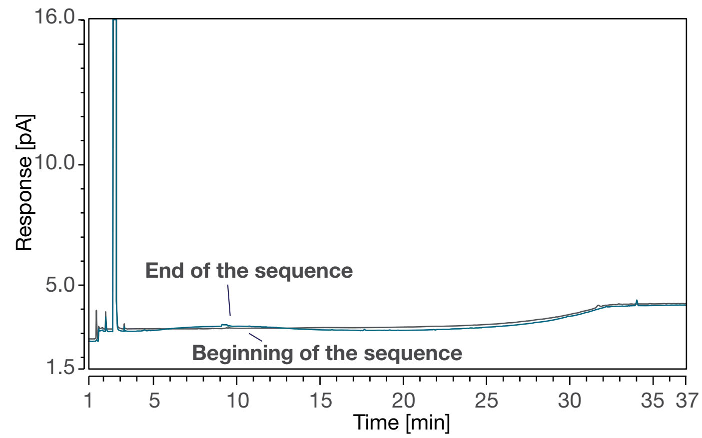

Figure 4. Carry-over was assessed by injecting a solvent blank at the beginning and end of the sequence

Conclusions

The results of these experiments demonstrate that the TriPlus RSH SMART autosampler in combination with the TRACE 1610 GC provides an ideal solution for laboratories dealing with biodiesel quality control assessment, and willing to deliver confident results for FAMEs characterization and quantitation.

- The automated sample handling capability of the TriPlus RSH SMART autosampler is beneficial for laboratories looking to reduce manual operations and associated risks of errors or cross contamination. The addition of the ISTD to the samples can be fully automated, also permitting, if needed, to scale down sample and ISTD volumes, reducing costs and waste.
- The precise built-in control of the TriPlus RSH SMART autosampler ensured consistent analytical performance, meeting and exceeding the acceptance criteria specified in the EN 14103 method, with the use of hydrogen as renewable carrier gas.
- The HeSaver-H_2Safer technology permits the safe use of carrier hydrogen as a renewable and cost-effective resource. Safety concerns related to the use of hydrogen are removed by limiting the maximum flow supplied to the inlet so that it becomes impossible to reach a hazardous concentration in the GC oven, even in case of leaks or column breakage, removing the need of hydrogen sensor installation.

References

1. ASTM D6584-21 Standard test method for determination of total monoglycerides, total diglycerides, total triglycerides, and free and total glycerin in B-100.
2. EN 14105 Fat and oil derivatives - Fatty acid methyl esters (FAMEs) - Determination of free and total glycerol and mono-, di-, triglyceride contents, September 2021.
3. EN 14110 Fat and oil derivatives - Fatty acid methyl esters (FAMEs) - Determination of methanol content, June 2020.
4. EN 14103 Fat and oil derivatives - Fatty acid methyl esters (FAMEs) - Determination of ester and linolenic acid methyl ester contents, December 2020.
5. Thermo Fisher Scientific, Application Note 001897: Biodiesel quality assessment: an automated approach for analysis of free and total glycerol content in biodiesel (B100), according to the EN 14105 and ASTM D6584 methods.
6. Thermo Fisher Scientific, Application Note 001898: Biodiesel quality assessment: characterization of residual methanol in finished biodiesel (B100) by headspace sampling according to EN 14110 standard.
7. EN 14214:2012, A2:2019 Liquid petroleum products - Fatty acid methyl esters (FAME) for use in diesel engines and heating applications - Requirements and test methods, October 2021.
8. Thermo Fisher Scientific, Technical Note 001218: Addressing gas conservation challenges when using helium or hydrogen as GC carrier gas.
9. Thermo Fisher Scientific, Application Note 001225: Automated and high-throughput derivatization for FAMEs analysis in vegetable oils and animal fats.

Appendix 1. TriPlus RSH SMART autosampler configuration for determination of methyl esters and linolenic acid methyl ester in biodiesel according to the EN 14103 standard and list of suggested consumables

Part number	TriPlus RSH SMART configuration	Qty
1R77010-2003	TriPlus RSH SMART Advanced Autosampler for liquid injections, regular rail(*) including: - one universal liquid syringe tool, for syringes of 0.5, 1.0, 5, 10, 25, 50 or 100 µL with a 57 mm needle length (P/N 1R77010-1007) - two 10 µL SMART syringes, 57 mm needle length, 26S gauge, cone needle type (P/N 365D0291-SM) - one tray holder (P/N 1R77010-1021) - three VT54 trays, for 54 vials/tray (P/N 1R77010-1023) - one standard washing station with 5 x 10 mL vials (P/N 1R77010-1029) (*) or equivalent TriPlus RSH base liquid / headspace configuration, in case of an existing instrument	1
1R77010-1020	Tool Releasing Station Stores one single syringe tool at a time. The tool is automatically parked and must be changed manually. One tool releasing station can be configured on each TriPlus RSH SMART Advanced autosampler	1
1R77010-1019	Alternatively, if the automated tool change capability is needed: Automatic Tool Change Station (ATC) Station Stores and changes automatically up to three syringe tools. Up to two ATC stations can be configured on each TriPlus RSH SMART Advanced autosampler	1
1R77010-1022	VT15 Vial Tray for 10/20 mL vials for up to 45 HS vials Sample tray for 15 vials of 10-20 mL. Vials are not included.	3
1R77010-1025	Alternatively, for extended sample capability: R60 Aluminum vial tray for 10/20 mL vials for up to 60 HS vials Sample tray for 60 vials of 10-20 mL. Vials are not included.	1

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Suggested consumables	Part number
10 µL Fixed Needle SMART syringe 57 mm needle length, 26S gauge, Cone needle type	365D0291-SM
Thermo Scientific™ SureSTART™ 10 mL Glass Screw Top Headspace Vials, Level 2 High-Throughput Applications	6ASV10-1
Thermo Scientific™ SureSTART™ 18 mm Precision Screw Caps, Level 3 High Performance Applications	6PMSC18-ST2
TRACE TR-BioDiesel column (F) 30 m × 0.25 mm × 0.25 µm	26AX142P
Thermo Scientific™ LinerGOLD™ Precision Liner, Split/Splitless with quartz wool	453A1255-UI

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Appendix 2. TriPlus RSH SMART autosampler and TRACE 1610 GC experimental conditions for analysis of FAMEs in biodiesel

TriPlus RSH SMART Autosampler parameters	
Injection volume (µL)	1
Fill strokes count	10
Pre-injection dwell time (s)	0
Post-injection dwell time (s)	0
Sample vial depth (mm)	40
Pre-injection wash cycles	0
Post-injection wash cycles	3
Post-injection wash solvent volume (µL)	6
Sample rinse cycles	2
Sample rinse volume (µL)	1
Syringe	10 µL, 57 mm, Ga 26s (P/N 365D0291-SM)

TRACE 1610 GC parameters	
iC-SSL HeSaver-H_2Safer	
Temperature (°C)	250
Liner	LinerGOLD Precision Split/Splitless with quartz wool (P/N 453A1255-UI)
Inlet module and mode	SSL upgraded to HeSaver-H_2Safer, split
Split flow (mL/min)	100
Septum purge flow (mL/min)	5, constant
Hydrogen delay (min)	0.2
Carrier gas, flow (mL/min)	H_2, 2.5
Oven temperature program	
Temperature (°C)	60
Hold time (min)	1.75
Rate (°C/min)	11.80
Temperature 2 (°C)	150
Rate (°C/min)	4
Temperature 3 (°C)	240
Hold time (min)	5.75
GC run time (min)	37.627
Ready delay (min)	1.3
FID	
Temperature (°C)	250
Air flow (mL/min)	350
H_2 flow (mL/min)	35
N_2 flow (mL/min)	40
Aquisition rate (Hz)	25
Analytical column	
TRACE TR-BioDiesel (F)	30 m × 0.25 mm × 0.25 µm (P/N 26AX142P)

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