Industrial

An automated and high-throughput approach for comprehensive analysis of biodiesel (B100) quality in compliance with EN and ASTM methods

Authors

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

[1]Thermo Fisher Scientific, Milan, IT

[2]Thermo Fisher Scientific, Bremen, DE

Keywords

Automated sample preparation, TriPlus RSH SMART, glycerin content, fatty acid methyl-esters (FAMEs), methanol, biodiesel, HeSaver-H_2Safer, gas chromatography, GC, flame ionization detection, FID, TRACE 1610, EN 14103, EN 14105, EN 14110, ASTM D6584

Introduction

Interest in alternative fuels produced from renewable sources such as vegetable oils has increased over the last few years, mainly due to their reduced environmental impact in comparison with conventional fossil fuels.

Biodiesel is a renewable and biodegradable alternative to mineral fuel, made from vegetable oils through a chemical process of transesterification using alcohol (Figure 1). The process results in two major products: free fatty esters (or biodiesel) and glycerin.

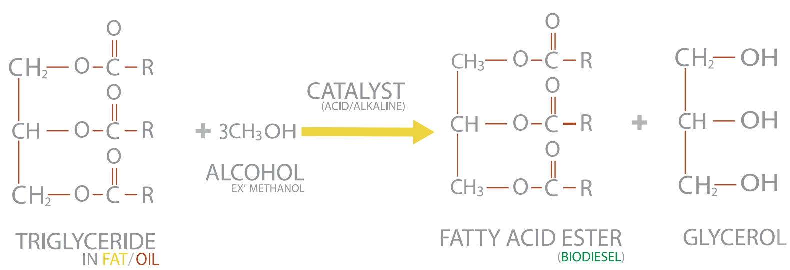

Figure 1. Trans-esterification reaction for biodiesel production

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 for commercialization and market acceptance since contaminants would compromise 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 intermediate product impurities in pure biodiesel (B100) using gas-chromatography (GC) coupled to flame ionization detection (FID):

- ASTM D6584 and EN 14105 for the determination of free and total glycerol and mono-, di-, triglyceride contents[1,2]
- EN 14110 for the determination of residual methanol[3]
- EN 14103 for the determination of total FAMEs (Fatty Acid Methyl Esters) and Linolenic Methyl Ester (C18:3)[4]

These methods require sample handling procedures before GC analysis, such as derivatization, dilution, and standard addition. Sample preparation, when executed manually, is a critical step in any analytical workflow as it may affect the overall accuracy and reliability of the results. It usually requires multiple steps resulting in time-consuming and expensive procedures that are prone to errors and cross-contamination.

Automated sample preparation represents a viable solution to overcome these challenges, delivering more accurate data for greater confidence, increasing sample throughput, and improving the analyst's safety by minimizing exposure to potentially harmful chemicals. The Thermo Scientific™ TriPlus™ RSH SMART autosampler provides advanced, built-in robotics that deliver high precision and accuracy, combined with the flexibility to fully automate routine sample handling operations, such as dilution or reagents addition, as well as more complex and customized sample preparation workflows.[5] The autosampler also provides an additional layer of reliability and confidence in the analytical results thanks to the automatic SMART syringe identification and usage tracking capabilities,[6] simplifying operations and offering a better management of the consumables.

The official methods required for a complete GC characterization of the biodiesel purity imply the use of different operative conditions and instrument setup, forcing the operator to prepare and analyze the same sample three times, wasting time and efforts. The robotic TriPlus RSH SMART autosampler[6] works more efficiently by automating sample preparation and GC analysis through a single automated and unattended workflow for all the methods.

Unprecedented productivity for comprehensive biodiesel analysis

To face the high throughput demand of analytical laboratories, a multi-method approach for the analysis of biodiesel samples can be achieved by operating a TriPlus RSH SMART autosampler in Clone Mode configuration, which allows it to serve two Thermo Scientific™ TRACE™ 1610 GC systems as two independent autosamplers. Different samples can be injected into two GCs—one dedicated to glycerol analysis, due to the high oven temperatures that have to be reached, and one dedicated to residual methanol and FAME content determination. The instrument configuration required for high throughput is reported in Figure 2 and detailed in the Appendix. The robotic autosampler is equipped to automate sample preparation for ASTM D6584 and EN 14105 methods as well as the addition of the internal standard for the EN 14110 method, ensuring a more precise quantitative analysis. Once the sample preparation is completed, the autosampler can automatically select and change the syringes to be used for sample injection thanks to the Automatic Tool Change (ATC) stations. This allows the analytical laboratory to assess the biodiesel quality by using one single platform with unattended operations, giving back to the user time for more valuable activities like data interpretation.

Data acquisition, processing, and reporting

For all the experiments, Thermo Scientific™ Chromeleon™ 7.3 Chromatography Data System (CDS) was used. The instrument control is fully integrated in the data system, ensuring a streamlined automated workflow from sample preparation to sequence set up, sample injection in one or two different GCs, and data acquisition, 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.

Key benefits

- The automated sample preparation capability of the TriPlus RSH SMART autosampler, serving one or two TRACE 1610 GCs when operating in Clone Mode, provides an ideal solution for laboratories performing quality control testing of biodiesel looking to increase operational efficiency and deliver confident results.

- The automated approach to calibration standards, sample derivatization, and internal standard addition allows for precise and reliable results, reduces the risk of errors, cross-contaminations and improves analysts' safety by limiting user's exposure to toxic chemicals.

- A single platform can be used for comprehensive biodiesel quality control according to the official methods with unattended operations and sample injection in two GCs, giving back to the user the time for more valuable activities like data interpretation.

The execution and the results of the biodiesel quality check methods for free and total glycerin (EN 14105 and ASTM D6584), FAME content (EN 14103) and residual methanol (EN 14110) with automated workflow including raw biodiesel sample preparation, calibration set up and GC analysis, are described in dedicated application notes, linked and briefly summarized in the next section.

The unique Clone Mode setup allows liquid injections to be performed independently and simultaneously on two GCs with one single autosampler, followed by headspace injections, automating in a single workflow the execution of each method presented in the dedicated application notes.

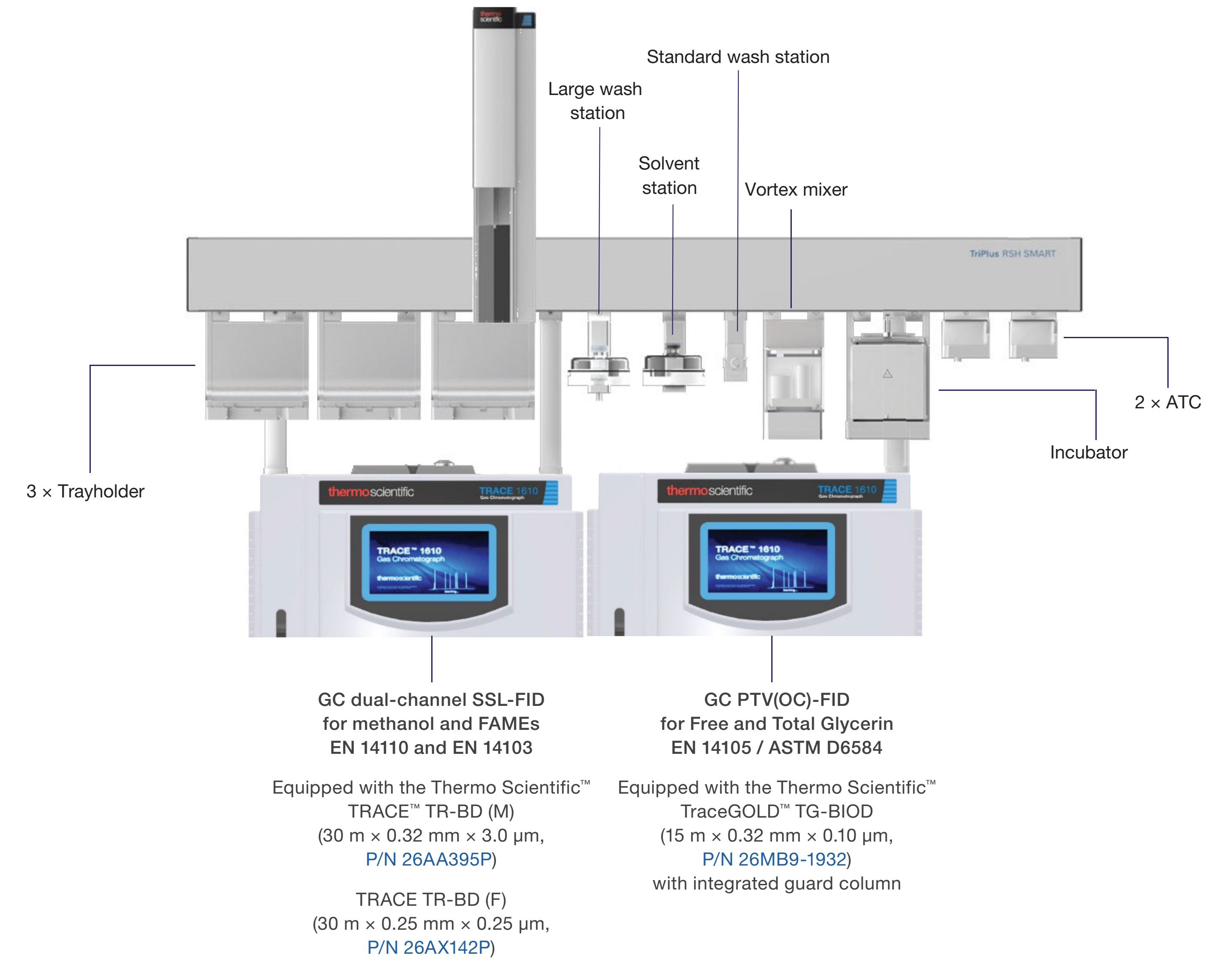

Figure 2. Biodiesel analyzer setup. TriPlus RSH SMART autosampler in Clone Mode configuration serves two TRACE 1610 GC systems as two independent autosamplers.

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

Application Note AN001897

The determination of residual unreacted glycerides and free glycerol in pure biodiesel (B100) is critical for the biodiesel quality before blending with mineral diesel, as high glycerin content can lead to injector clogging and formation of deposits in injector nozzles, pistons, and valves. In particular, the ASTM D6751[7] and the EN 14214[8] standards set the quality thresholds for total glycerin content to 0.24% and 0.25% by mass, respectively.

The GC analysis of free glycerol and glycerides in biodiesel requires a derivatization step with *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA) so that the target analytes are transformed into more volatile and stable silyl derivatives prior to injection. The sample preparation procedure is complex and time-consuming, and it exposes the user to hazardous chemicals. The possibility to automate manual steps through a robotic autosampler offers increased sample throughput with unattended operations, enhanced data precision and reliability by removing possible human errors, and less exposure to toxic reagents. The two methods differ only for the use of the internal standards (Figure 3) and the quantitative calculation.

ASTM D6584

1. Weigh 100 mg sample
2. Calibration curve preparation
3. ISTD 1 addition (Butanetriol, 100 µL)
4. ISTD 2 addition (Tricaprin, 100 µL)
5. MSTFA addition (100 µL)
6. Vortex (6 s, 1,200 rpm)
7. Wait (15 min)
8. n-heptane addition (8 mL)
9. Vortex (6 s, 1,200 rpm)

ASTM D6584 calibration curve preparation

Commercial standard solution	Calibration curve levels (stock standard, µL)				
	1	2	3	4	5
Glycerin solution in pyridine (0.5 mg/mL)	10	30	50	70	100
Monoolein solution in pyridine (5 mg/mL)	20	50	100	150	200
1,3-Diolein solution in pyridine (5 mg/mL)	10	20	40	70	100
Triolein solution in pyridine (5 mg/mL)	10	20	40	70	100

ASTM D6584 calibration ranges

Compound	Calibration curve levels (mg/mL)				
	1	2	3	4	5
Glycerin	0.005	0.015	0.025	0.035	0.050
Monoolein	0.10	0.25	0.50	0.75	1.00
1,3-Diolein	0.05	0.10	0.20	0.35	0.50
Triolein	0.05	0.10	0.20	0.35	0.50

EN 14105

1. Weigh 100 mg sample
2. Calibartion curve preparation
3. ISTD 1 addition (Butanetriol, 80 µL) 200 µL)*
4. Pyridine addition (200 µL)*
5. MSTFA addition (cal curve=150 µL, samples=200 µL)
6. Vortex (6 s, 1,200 rpm)
7. Wait (15 min)
8. n-heptane addition (8 mL)
9. Vortex (6 s, 1,200 rpm)

* Reagent is added to samples only

EN 14105 calibration curve preparation

Stock standard solution	Calibration curve levels (stock standard, µL)			
	1	2	3	4
Glycerin solution in pyridine (0.5 mg/mL)	10	40	70	100

EN 14105 calibration ranges

Compound	Calibration curve levels (mg/mL)			
	1	2	3	4
Glycerin	0.005	0.02	0.035	0.050

Figure 3. Schematic of automated sample preparation workflows for analysis of free and total glycerol content in biodiesel according to ASTM D6584 and EN 14105 methods. Calibration curves were prepared according to the tables.

The use of the dedicated TraceGOLD TR-BIOD column for the chromatographic separation ensures reliable and reproducible performance with low bleed even at elevated temperatures, making this column ideal for the analysis of free and total glycerol content according to ASTM D6584 and EN 14105 methods. Hydrogen was used as carrier gas, providing efficient chromatographic separation combined with short GC run times (Figure 4).

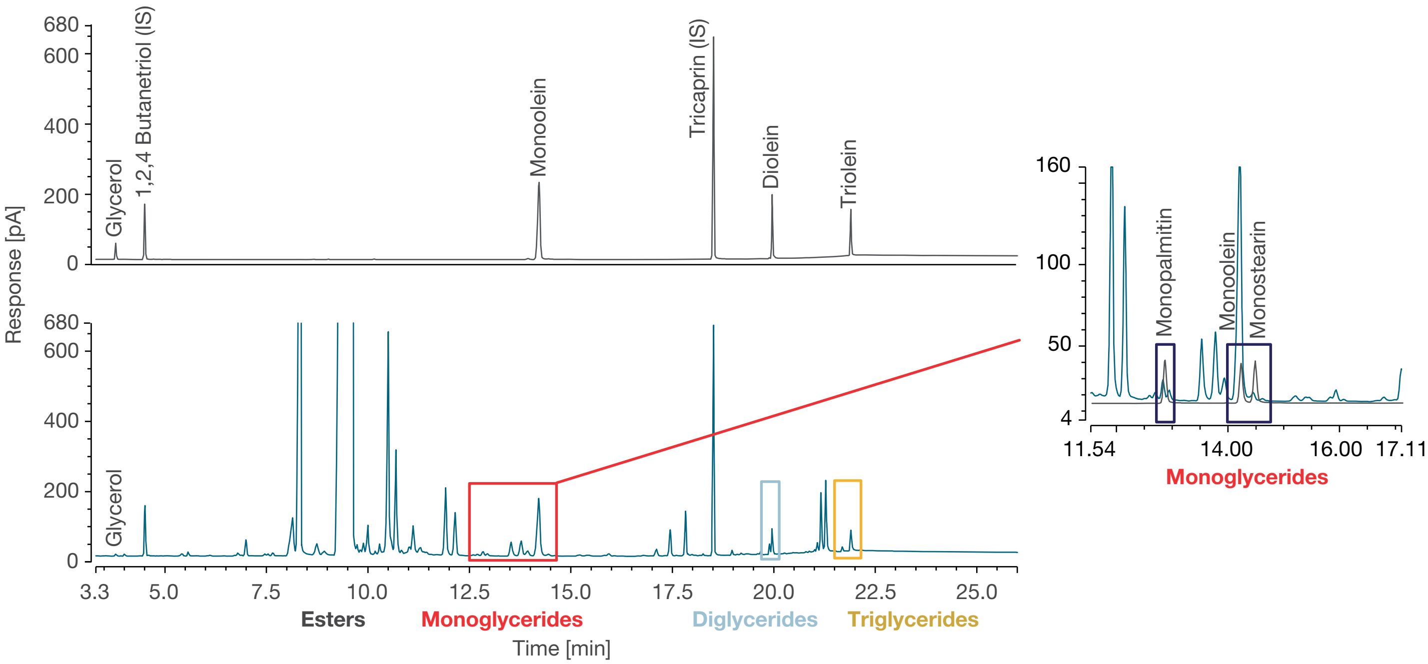

Figure 4. Example of typical chromatograms for reference standard mix (gray trace) and unknown biodiesel sample (blue trace). The inset shows the monoglycerides elution window with overlay of the monoglycerides standard solution (gray trace).

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

Application Note AN001899

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 number 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,[8] 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.

The dedicated TRACE TR-BioDiesel (F) GC 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 (Figure 5). In these experiments, hydrogen was used as carrier gas providing a renewable and efficient alternative to helium. The use of the Thermo Scientific™ iConnect™ SSL injector upgraded with the Thermo Scientific™ HeSaver-H_2Safer™ technology provides limited 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 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.

Sample handling can be automated with the TriPlus RSH SMART autosampler, which permits the automated addition of the ISTD to the samples using a dedicated prep cycle, limiting the manual operations to just sample weighing. Automated procedures also offer the possibility to scale down sample and ISTD volumes, maintaining highly precise operations.

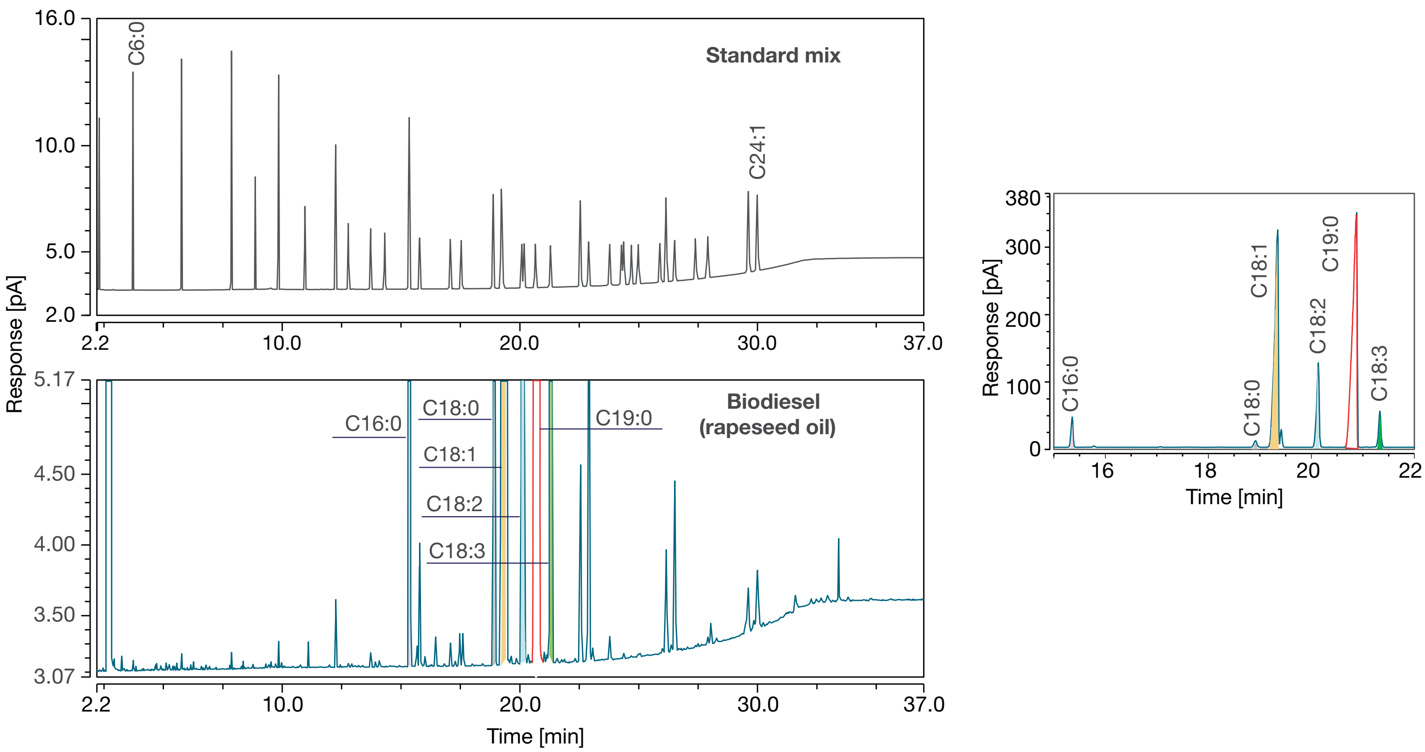

Figure 5. Typical chromatograms for 37-component FAME mix and biodiesel sample from rapeseed oil. The peak integration area specified in the method is from C6:0 to C24:0. The spiked ISTD (C19:0) in the biodiesel sample is marked in red. The inset shows a zoomed-out view of the individual FAMEs typically found in B100 obtained from rapeseed oil.

Biodiesel quality assessment: characterization of residual methanol in finished biodiesel (B100) by headspace sampling according to EN 14110 standard

Application Note AN001898

Methanol is commonly used as transesterification agent in biodiesel production, and therefore, residual methanol may be found as an impurity in the finished product. Monitoring residual methanol is a matter of safety since even small amounts can reduce the flash point of biodiesel, potentially affecting the safe operation of fuel pumps and lifetime of seals and elastomers, resulting in poor combustion. Quality specifications for methanol content are stated in the EN 14214[8] with an allowed amount of less than 0.2% m/m. Methanol content in finished B100 is determined according to the EN 14110 standard using gas chromatography (GC) coupled to headspace (HS) as a sampling technique followed by detection using a flame ionization detector (FID).

The dedicated TRACE TR-BioDiesel (M) GC column is specific for the analysis of residual methanol according to the EN 14110 standard, ensuring reliable and reproducible results. The use of hydrogen as carrier gas in combination with the HeSafer-H_2Safer technology offers a sustainable and cost-saving solution without safety concerns, thanks to the limited maximum flow supplied to the inlet, with no need for H_2 sensor installation in the GC oven.

The TriPlus RSH SMART autosampler can be used for the automated addition of the internal standard before sample incubation for headspace analysis, by using a dedicated 10 µL syringe. As stated in the method, the addition of the internal standard is preferred only when a small number of samples is analyzed and when automatic headspace equipment is not available. In general, the use of internal standard is recommended for quantitative analysis to increase the accuracy and to compensate for matrix effect.

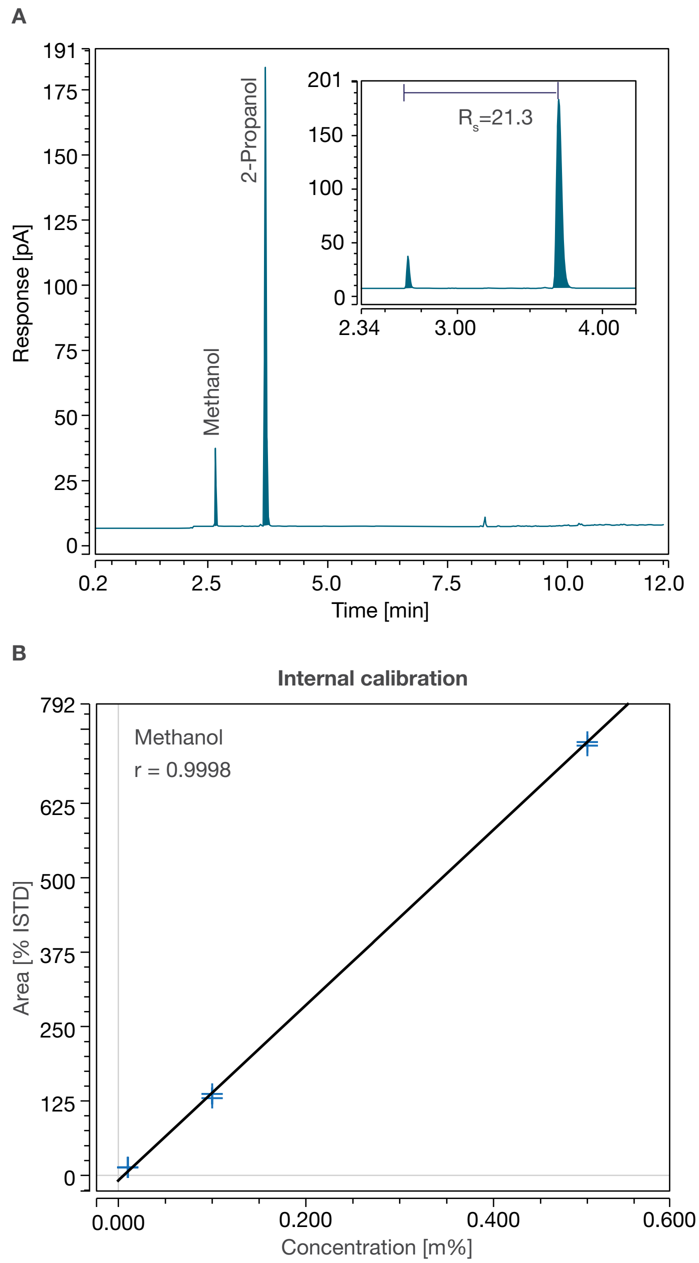

Figure 6. Analysis of biodiesel. (A) Typical headspace chromatogram for a biodiesel (B100) sample spiked with 2 µL of internal standard. The inset shows a zoomed view of the peaks with the achieved chromatographic resolution annotated. (B) Calibration curve by using internal calibration, with r value exceeding the minimum method requirement of 0.95.

References

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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, Guide to automated sample preparation for GC and GC-MS: EB000396
6. Thermo Fisher Scientific, TriPlus RSH SMART robotic sampling system brochure: BR52235-EN 0921C
7. ASTM D6751-20a Standard Specification for Biodiesel Fuel Blend Stock (B100) for Middle Distillate Fuels.
8. 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.

Appendix

Part number	TriPlus RSH SMART configuration for comprehensive analysis of biodiesel	Qty
1R77010-2004	TriPlus RSH SMART Advanced for liquid injections, extended 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
1R77010-1019	Automatic Tool Change Station (ATC) Station Up to two ATC stations can be configured on each TriPlus RSH SMART Advanced autosampler	2
1R77010-1031	Solvent Station 3 × 100 mL solvent bottles Bottles with seal and caps are included	1
1R77010-1030	Large Wash Station for 2 × 100 mL solvent bottles and one waste position Bottles with seal and caps are included	1
1R77010-1033	Vortexer Module Suitable for 2-, 10-, or 20-mL vial	1
1R77010-1195	Headspace upgrade kit including: • one headspace tool for 2.5 mL syringe (P/N 1R77010-1013) • one tray holder (P/N 1R77010-1021) • one vial tray R60 aluminum tray for 10/20 mL vials (P/N 1R77010-1025) one Incubator/Agitator (P/N 1R77010-1032) • two HT 2.5 mL GT SMART syringes (P/N 365L2321-SM)	1
1R77010-1021	Tray holder	1
1R77010-1022	VT15 Vial Tray for 10/20 mL vials Sample tray for 15 vials of 10-20 mL. Vials are not included Mandatory for ASTM D6584 and EN 14105 methods	3
1R77010-1025	R60 aluminum tray for 10/20 mL vial	1
1R77010-1022	Alternative to the R60 vial tray: VT15 Vial Tray for 10/20 mL vials Sample tray for 15 vials of 10-20 mL. Vials are not included	3
1R77010-1008	Universal liquid syringe tool, for syringes of 5 µL, 10 µL, 25 µL, 50 µL, or 100 µL with a needle length of 85 mm SMART syringes not included	1
1R77010-1007	Universal liquid syringe tool, for syringes of 0.5, 1.0, 5, 10, 25, 50, or 100 µL with a needle length of 57 mm SMART syringes not included	2
1R77010-1011	Universal Liquid Syringe Tool for a 10,000 µL syringe with a needle length of 57 mm SMART syringes not included	1

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