Application note | 001898

Industrial

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

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Keywords

Automated sample preparation, HeSaver-H_2Safer, TriPlus RSH SMART, headspace, methanol, biodiesel, B100, gas chromatography, GC, flame ionization detection, FID, EN 14110

Goal

The aim of this study is to demonstrate the suitability of the Thermo Scientific™ TRACE™ 1610 GC series combined with the Thermo Scientific™ TriPlus™ RSH SMART autosampler for characterization of residual methanol in biodiesel according to the EN 14110:2020 method.

Introduction

Fuel-grade biodiesel is made from natural oils through a chemical process of transesterification with alcohol, resulting in two major products: free fatty esters (or biodiesel) and glycerin. When methanol is used for the transesterification process, methyl esters of fatty acids are generated. Once separated from glycerin, biodiesel can be blended with petroleum diesel in various concentrations, typically 5% (B5) up to 20% (B20), to be used in diesel engines 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) and flame ionization detection (FID):

- ASTM D6584 for the determination of total residual monoglyceride, diglyceride, triglyceride, and free and total glycerin content[1]
- EN 14105 for the determination of 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)[4]

This application note focuses on the method EN 14110. Refer to AN001897[5] for results on methods EN 14105/ASTM D6584 and to AN001899[6] for results on method EN 14103.

EN 14110

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[7] 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).

Experimental

In this study, a TriPlus RSH SMART Advanced autosampler was coupled to a TRACE 1610 GC equipped with a Thermo Scientific™ iConnect™ split/splitless (iC-SSL) injector upgraded to work in HeSaver-H_2Safer mode and a Thermo Scientific™ iConnect™ flame ionization detector (iC-FID). The TriPlus RSH SMART autosampler was configured as shown in Figure 1. A dedicated prep cycle[8] was used for automated addition of the internal standard before sample incubation, 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. A detailed description of the autosampler configuration, including a complete list of suggested consumables, is reported in Appendix 1.

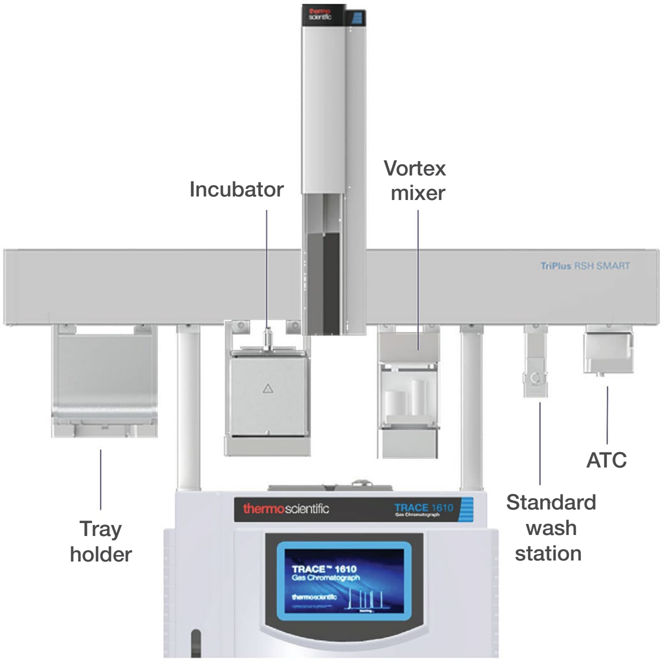


Figure 1. TriPlus RSH SMART autosampler configuration for automated addition of internal standard prior to headspace sampling

The Thermo Scientific™ HeSaver-H_2Safer™ carrier gas saving technology[9] offers an innovative and smart approach to dramatically reduce carrier gas consumption, especially during GC operation. One of the key benefits of the HeSaver-H_2Safer mode is that the method used with the standard SSL injector remains unchanged without need for re-optimization.

Chromatographic separation was achieved on a Thermo Scientific™ TRACE™ TR-BioDiesel (M) GC column (30 m × 0.32 mm × 3.0 µm, P/N 26AA395P). This column is specific for the analysis of residual methanol according to the EN 14110 standard, ensuring reliable and reproducible results. In this study, the use of hydrogen as carrier gas was preferred as it is a renewable and cheaper gas compared to helium, with no impact on FID detector response. In addition, the use of HeSaver-H_2Safer technology removes safety concerns related to the use of hydrogen by limiting the maximum flow supplied to the inlet even in case of leaks in the oven or column breakage, thus eliminating the need to install a hydrogen sensor. Detailed experimental conditions are reported in Appendix 2.

Standard preparation

Methanol (LC/MS grade, purity 99.9%, Fisher Scientific P/N 10031094) was diluted in the biodiesel blank certified reference material (CRM B100), used as the reference FAME sample, to obtain the calibration levels according to the EN 14110 standard (Table 1). 2-Propanol (HPLC/GC grade, purity 99.9%, Fisher Scientific P/N 15950721) (IS, 2 µL) was automatically added as Internal Standard to each calibration solution and sample by using the TriPlus RSH SMART autosampler.

Table 1. Schematic of calibration curve preparation of methanol in reference FAME sample (CRM B100)

Calibration solution	Methanol concentration (% w/w)	Spiking solution	Spiked volume (mL)	Reference FAMEs (mL)
A	0.5	Methanol	0.142	25
B	0.1	Solution A	5	20
C	0.01	Solution B	1	9

Sample preparation

An aliquot of unknown biodiesel sample (2 mL) was transferred into 20 mL screw top headspace vials (P/N 6ASV20-1, caps P/N 6PMSC18-ST2) and seated in the autosampler tray. The addition of the ISTD (2-propanol) was automatically performed by the TriPlus RSH SMART autosampler.

Instrument control and data acquisition

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 from sample preparation to sequence setup, sample injection, 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.

Results and discussion

Chromatography

The headspace sampling allows for fast extraction of volatile analytes from complex non-volatile matrix. This technique is therefore particularly convenient to extract the residual methanol from biodiesel samples. Chromatographic resolution between methanol and 2-propanol was automatically calculated from Chromeleon CDS by using the European Pharmacopeia (EP) formula, as prescribed in the EN 14110:2020 method. The reliable performance of the TRACE TR-BioDiesel (M) column exceeded by far the minimal required chromatographic resolution ($R_s \geq 1.5$) between methanol and 2-propanol and provided Gaussian peak shapes, as demonstrated in Figure 2.

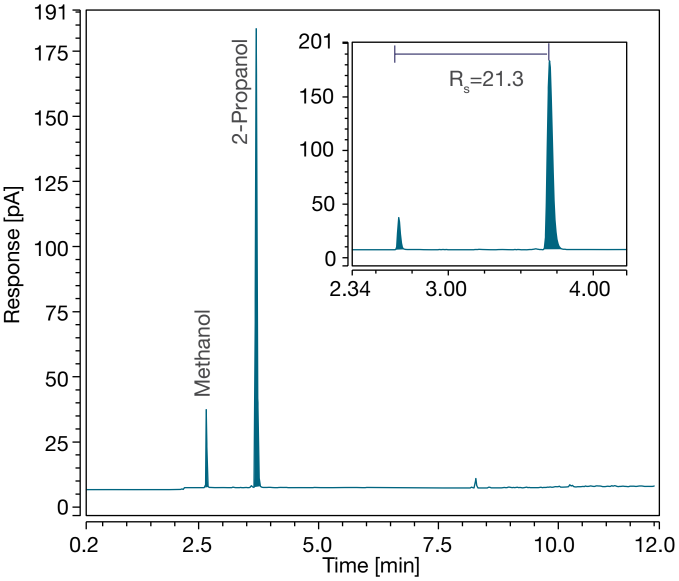


Figure 2. Typical headspace chromatogram for a biodiesel (B100) sample spiked with 2 µL of internal standard. The inset shows a zoomed view of the peaks with the achieved chromatographic resolution annotated.

Calibration

Linearity of the response was assessed by running a three-point calibration curve as detailed in the EN 14110 method, with each calibration point prepared in duplicate. The calibration curve was plotted using both the internal and the external calibration. The correlation coefficients (r) met and exceeded the minimum method requirement of 0.95, being 0.9998 and 0.9995, respectively, as shown in Figure 3. When internal calibration is used, the method requires checking the linearity by calculating the calibration factor F (Equation 1) for each of the calibration levels, and verifying that their coefficient of variation (CV%) is below 15%. In this study, the CV% of the calibration factors was 4.7%, well below the acceptance limit.

Equation (1) $F = (C_m * S_i) / (C_i * S_m)$

C_m = methanol content in the calibration solution, in %w/w
S_i = peak area of 2-propanol
C_i = 2-propanol content in the calibration solution, in %w/w
S_m = peak area of methanol

Quantitative performance was evaluated by running a sequence containing a total of 45 samples, including blank samples, calibration standards, raw biodiesel, and quality control samples (QCs). The quality control samples consisted of the CRM B100 spiked at 0.1% w/w. A baseline chromatogram was acquired at the beginning and end of the sequence to evaluate possible carry-over (Figure 4). Methanol content in unknown biodiesel samples was well within the allowed limits, with calculated amounts of 0.0032%.

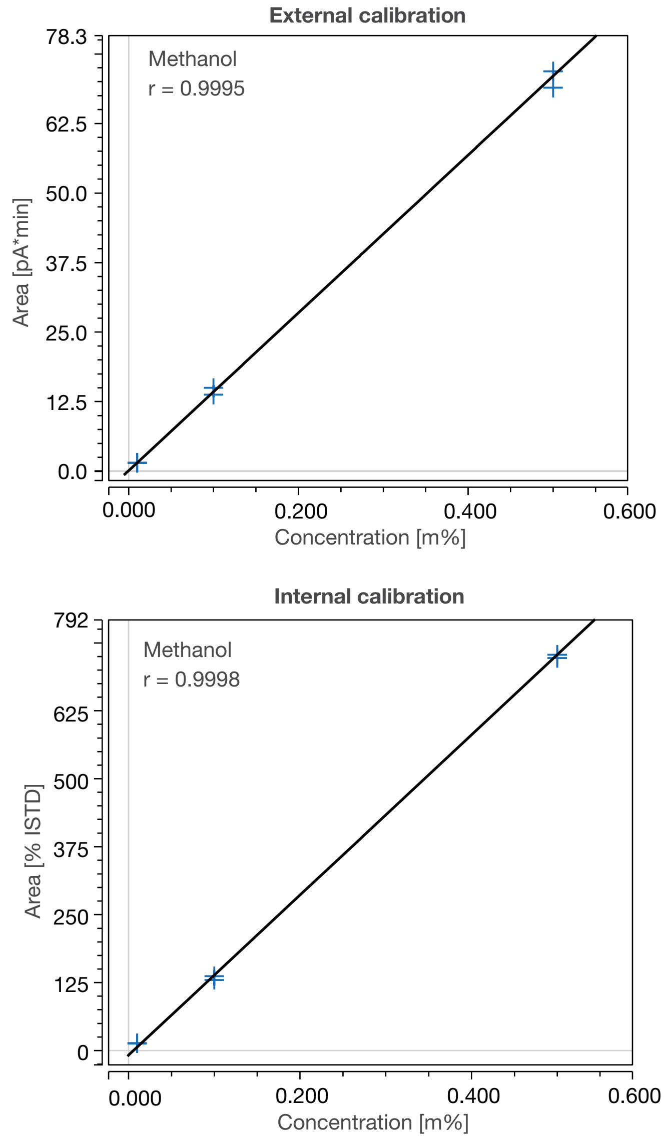


Figure 3. Calibration curves plotted by using external and internal calibration. In both cases, r value met and exceeded the minimum method requirement of 0.95.

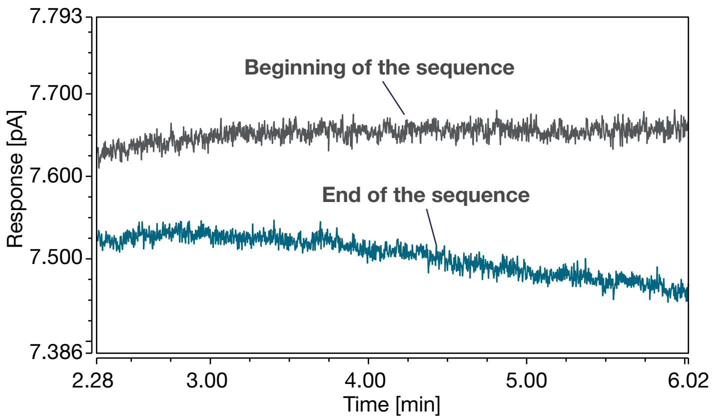
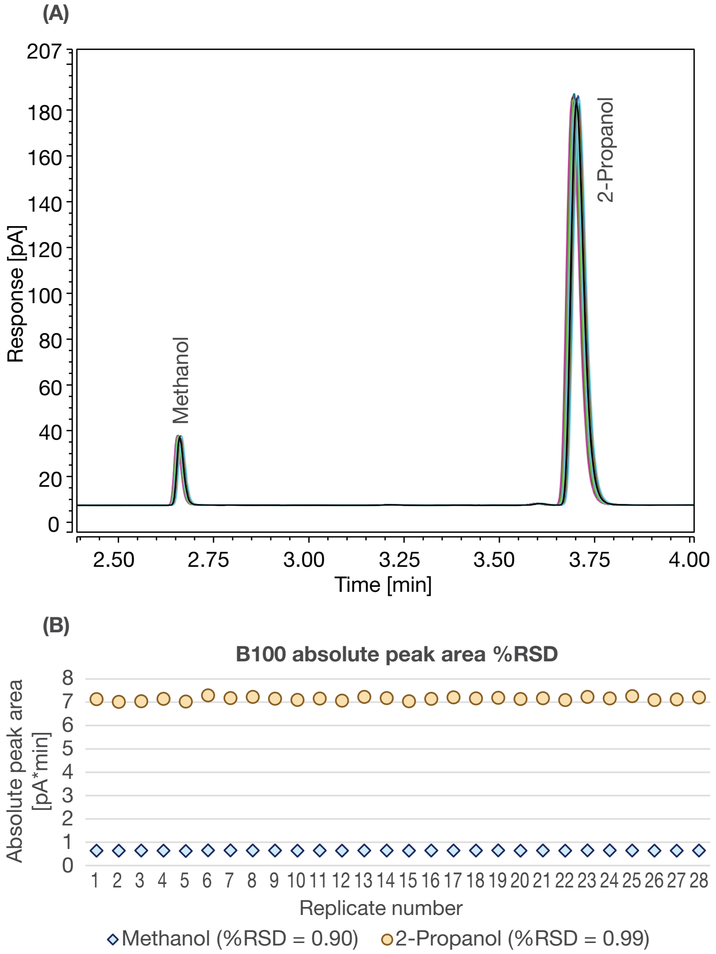


Figure 4. Carry-over was assessed by acquiring a baseline chromatogram at the beginning and end of the sequence.

Recovery

Calculated amounts for n=28 spiked biodiesel QCs were within 3% of the spiked concentration, with % recovery ranging from 100 to 103%. Overlaid chromatograms of biodiesel QC samples show the excellent repeatability with absolute peak areas %RSD < 1% (Figure 5).



(C)

QC number	Methanol peak area (pA*min)	2-Propanol peak area (pA*min)	Spiked amount (%)	Calc. amount (%)	Calc. recovery
1	14.5445	7.0036	0.1	0.1022	102
2	14.5989	7.0162	0.1	0.1026	103
3	14.5813	6.9585	0.1	0.1024	102
4	14.5596	7.0416	0.1	0.1023	102
5	14.5405	7.0694	0.1	0.1022	102
6	14.5425	6.9781	0.1	0.1022	102
7	14.1768	6.9904	0.1	0.0996	100

Figure 5. Overlaid headspace chromatograms (n=28) of biodiesel QC samples are shown in (A), and repeatability of the peak areas in (B). The absolute peak area %RSD for the analyzed samples and the calculated recoveries for QC samples analyzed within the same sequence are reported in the table (C).

Repeatability

Repeatability was assessed according to the EN guidelines. The difference between two test results obtained by the same operator with the same apparatus under constant operating conditions on identical test material must not exceed the r value, calculated according to Equation 2. This requirement was verified over n=28 sample injections, as reported in Figure 6.

Equation (2) $r = 0.056\ X + 0.001$

X = mean of the two results being compared

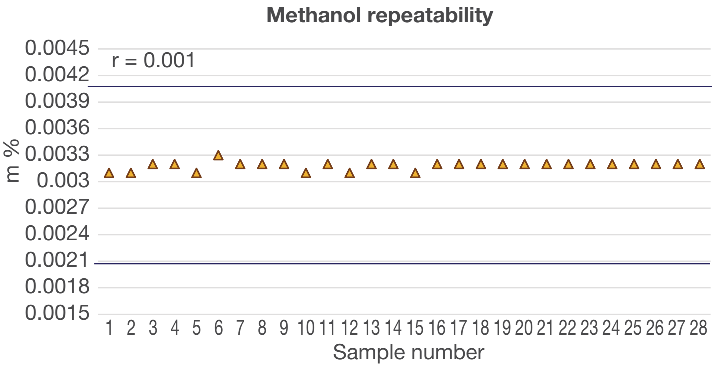


Figure 6. Repeatability calculated according to the EN 14110 standard by calculating the difference among n=28 replicates of the same sample

Conclusions

The results of these experiments demonstrate that the automated sample preparation capability of the TriPlus RSH SMART autosampler offers an ideal solution for laboratories working to ensure consistent quality of biodiesel and looking to improve productivity with confident results.

- The addition of the internal standard can be automated for more precise quantitative analysis by using a dedicated prep cycle.
- The advanced technology of the TriPlus RSH SMART autosampler ensures precise control of volumes and flows, delivering more consistent results, and meeting and exceeding the minimum acceptance criteria applied for biodiesel quality control.
- The use of hydrogen as renewable and cheaper carrier gas compared to helium, offers a more sustainable solution. The HeSaver-H_2Safer technology removes the safety concerns related to the use of hydrogen and eliminates the need of a hydrogen sensor in the GC oven, as it becomes impossible to reach a hazardous concentration in the oven, even in case of leaks or column breakage.

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 001899: Biodiesel quality assessment: determination of esters and linolenic acid methyl ester content in biodiesel (B100) by GC-FID, according to EN 14103.
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, Guide to automated sample preparation for GC and GC-MS: EB000396
9. Thermo Fisher Scientific, Technical Note 001218: Addressing gas conservation challenges when using helium or hydrogen as GC carrier gas.

Appendix 1. TriPlus RSH SMART autosampler configuration and suggested consumables for automated addition of internal standard and headspace sampling for determination of residual methanol in biodiesel

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
1R77010-1019	Automatic Tool Change Station (ATC) Station Up to two ATC stations can be configured on each TriPlus RSH SMART Advanced autosampler	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
1R77010-1022	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

For more details about orders and quotations, please contact your Thermo Fisher Scientific sales representative.

Suggested consumables	Part number
10 µL Fixed Needle SMART syringe 57 mm needle length, 26S gauge, Cone needle type	365D0291-SM
2.5 mL Gas-Tight HT SMART Headspace syringe	365L2321-SM
Thermo Scientific™ SureSTART™ 20 mL Glass Screw Top Headspace Vials, Level 2 High-Throughput Applications	6ASV20-1
Thermo Scientific™ SureSTART™ 18 mm Precision Screw Caps, Level 3 High Performance Applications	6PMSC18-ST2
TRACE TR-BioDiesel column (M) 30 m × 0.32 mm × 3.0 µm	26AA395P
SSL Direct Straight Liner for HS/SPME Deactivated, 1.2 mm ID × 6.3 mm OD × 78.5 mm length	453A1335

Appendix 2. TriPlus RSH SMART autosampler and TRACE 1610 GC parameters for the EN 14110 method

TriPlus RSH SMART Autosampler parameters	
Headspace injection volume (µL)	500
Incubation temperature (°C)	60
Incubation time (min)	15
Agitation speed (rpm)	500
Sample draw (mL)	0.5
Sampling depth mode, depth (mm)	Custom, 35
Syringe temperature (°C)	75
Enable pre-filling	Yes
Filing strokes volume (mL)	1.2
Filling stokes counts	5
Filling delay (s)	30
Pre-injection syringe flush	Disabled
Post-injection syringe flush (s)	60
Filling speed (mL/min)	6
Injection speed (mL/min)	25
Injection depth (mm)	45
Penetration speed (mm/s)	25
Pre-injection delay (s)	3
Post-injection delay (s)	10
Syringe	2.5 mL, Gas-Tight HT SMART (P/N 365L2321-SM)

TRACE 1610 GC parameters	
iC-SSL HeSaver-H_2Safer	
Temperature (°C)	150
Liner	SSL Direct Straight Liner (P/N 453A1335)
Inlet module and mode	SSL upgraded to HeSaver-H_2Safer, split
Split flow (mL/min)	50
Septum purge flow (mL/min)	5, constant
Hydrogen delay (min)	0.2
Carrier gas, pressure (kPa)	H_2, 39.80
Oven temperature program	
Temperature (°C)	50
Hold time (min)	6
Rate (ºC/min)	50
Temperature 2 (°C)	200
Hold time (min)	3
GC run time (min)	12
Ready delay (min)	1.4
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 (M)	30 m× 0.32 mm × 3.0 µm (P/N 26AA395P)

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