Application Note: 10215

Determination of Free and Total Glycerin in Pure Biodiesel (B100) by GC in Compliance with EN 14105

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Key Words

- TRACE GC Ultra
- Biodiesel (B100)
- EN 14105
- Free and Total Glycerin
- True Cold On-column Injector

Introduction

Interest in biodiesel as a clean-burning alternative fuel produced from renewable sources such as vegetable oils has increased tremendously over the last few years, mainly due to its reduced environmental impact in comparison with conventional petro-diesel. Biodiesel can be used as a pure fuel or blended at any level with petroleum diesel. 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 standards indicate the maximum allowable concentrations of contaminants in pure (B100) finished product, along with other chemical-physical properties necessary for a safe and satisfactory engine operation.

Since biodiesel is produced by transesterification of the raw oil with methanol in the presence of a catalyst (usually sodium hydroxide), the resulting product may contain not only the desired methyl esters but also unreacted starting material (triglycerides) and residual methanol. Traces of glycerin, obtained as a valuable by-product and separated from biodiesel during the production process, can be also found in the final mix. Finally, traces of mono- and di-glycerides formed as intermediates can be present in biodiesel.

Gas chromatography 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) [3,4]
- **EN 14105/ASTM D6584**: Determination of Free and Total Glycerine [5,6,7]
- **EN 14110**: Determination of residual Methanol [8,9]

Comprehensive gas chromatography solutions that comply with each of these methods have been developed, based on the Thermo Scientific TRACE GC Ultra™ and the versatile Thermo Scientific TriPlus™ autosampler (Figure 1). This application note relates specifically to the use of the TRACE GC Ultra and TriPlus autosampler for use in the determination of free and total glycerin in biodiesel, according to EN 14105.

Free and bonded glycerin content is an indicator of the biodiesel quality. Low levels of total glycerin ensure high conversion of the oil, while high levels of glycerin and glycerides can cause injector deposits, clogged fuelling systems, and poor cold weather operation. The determination of glycerin levels provides verification that the free glycerin, mono-glycerides, di-glycerides, tri-glycerides, and total glycerin contents in B100 are lower than the limits shown in Table 1, in accordance with the specifications reported in EN 14214:2003.

Figure 1: TRACE GC Ultra with TriPlus AS autosampler

The described method is suitable for biodiesel produced from rapeseed, soybean, sunflower oil, and is not suitable for biodiesel produced from or containing coconut and palm kernel oil, due to the presence of overlapping peaks.

Compound	Max % m/m (EN 14214)
Free Glycerin	0.02
Mono-glycerides	0.8
Di-glycerides	0.2
Tri-glycerides	0.2
Total Glycerin *	0.25

** Total Glycerin is calculated as follows: TG = G + 0.255M + 0.146D + 0.103T, where TG = Total Glycerin, G = Free Glycerin, M = Mono-glycerides, D = Di-glycerides, T = Tri-glycerides.*

Table 1: Free and total glycerin specifications according to EN 14214:2003

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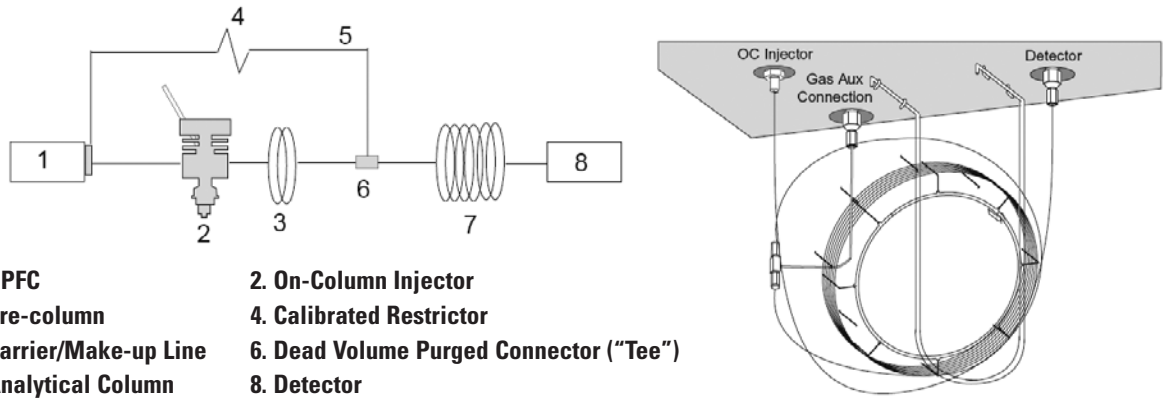


1. DPFC **2. On-Column Injector**
3. Pre-column **4. Calibrated Restrictor**
5. Carrier/Make-up Line **6. Dead Volume Purged Connector ("Tee")**
7. Analytical Column **8. Detector**

Figure 2: Leak free high T purged connector

Methods

Instrumentation and Reagents

The analysis of glycerin, mono, di and triglycerides by gas chromatography (GC) requires a non-discriminative injection system able to transfer both volatile and heavy compounds without discrimination or degradation. A TRACE GC Ultra equipped with a true cold On-column inlet and a flame ionization detector (FID), automated by a TriPlus Autosampler for liquids was used, controlled through the Thermo Scientific Chrom-Card data system. The true cold On-column injector offered on the TRACE GC Ultra is a permanently cold system, able to prevent discrimination of the heavier fraction and to eliminate any risk of degradation of labile components like triglycerides, thus granting excellent recovery and proven sample integrity.

The analytical column used is a non-polar Thermo Scientific TRACE™ TR-BIODIESEL(G), 10 m, 0.32 mm ID, 0.1 µm f.t. This column is designed to provide excellent performance for this high temperature GC method, featuring enhanced mechanical robustness at high oven temperature, and thus prolonged lifetime. A 1 m x 0.53 mm ID pre-column is used, connected to the column by a leak-free high T purged connection.

A low dead volume leak-free metal tee has been specifically conceived for a reliable connection between the guard column and the analytical column at high temperature operation, preventing from the use of normal glass press-fit unions. Such a connector has proven to stay leak-free even with extremely large and frequent oven temperature variations (Figure 2).

Calibration is achieved by the use of two internal standards – 1,2,4-butanetriol (IS1) for glycerine and tricaprin (IS2) for mono-, di- and tri-glycerides, and four reference compounds – glycerin, mono-olein, di-olein and tri-olein.

Because glycerin and mono- and di-glycerides are polar and high boiling components, they must be derivatized to increase their volatility and reduce activity before injection into the GC. The method requires derivatization with MSTFA (N-methyl-N-trimethylsilyltrifluoroacetamide) in pyridine, which transforms these compounds into more volatile silylated derivatives.

Below is a list of required reagents:

- MSTFA (N-methyl-N-trimethylsilyltrifluoroacetamide)
- *n*-Heptane
- Pyridine
- 1,2,4-Butanetriol – internal standard solution 1, 1 mg/mL in pyridine (IS1)
- 1,2,3-Tricaproylglycerol (tricaprin) - internal standard solution 2, 8 mg/mL in pyridine (IS2)
- Reference materials: glycerol (glycerin), 1-mono-oleoylglycerol (monoolein), 1,3-di-oleolglycerol (diolein), 1,2,3-tri-oleoylglycerol (triolein)
- Mono-glycerides check mix (monopalmitin, monostearin and monoolein), in pyridine

Sample Preparation

Four commercially available calibration solutions were used, containing glycerin, monoolein, diolein, triolein, butanetriol (internal standard IS1), and tricaprin (internal standard IS2) at concentrations specified in the EN 14105 method. 100 µL of the derivatization agent, MSTFA, were added to each calibration solution in a 10 mL vial, then hermetically sealed and shaken vigorously. After 15 minutes, 8 mL of *n*-heptane were added to each calibration mix. These final reaction mixtures were directly injected into the gas chromatograph.

For each biodiesel sample or standard to be analyzed, approximately 100 mg of homogenized sample were accurately weighed (± 0.1 mg) in a 10 mL vial; then 80 µL of IS1, 100 µL of IS2 and 100 µL of MSTFA were added to the sample vial, which was hermetically sealed and shaken vigorously. After 15 minutes, 8 mL of *n*-heptane were added. For analysis, 1 µL of the reaction mixture was automatically injected into the gas chromatograph, following the instrumental conditions described in Table 2. The same preparation procedure was followed for the mono-glycerides check mix.

Analytical Parameters

Table 2 includes selected method parameters for the TRACE GC Ultra and the TriPlus AS autosampler. These settings were used for all sample analyses.

TRACE GC Ultra	
Injector	True cold On-column
Carrier Gas	Helium, 3 mL/min for 12 min, then ramped to 5 mL/min at 0.5 mL/min2
FID	380 °C
Oven Program	80 °C (1 min) to 180 °C @ 15 °C/min, then to 230 °C @ 7 °C/min, then to 365 °C (4 min) @ 10 °C/min
TriPlus Autosampler	
Syringe Size	10 µL with 80 mm needle
Injected Volume	1 µL

Table 2: Selected instrument and method parameters for the TRACE GC Ultra and TriPlus AS

Results and Discussion

System Calibration

The system was calibrated by analyzing the 4-component calibration mix at four different concentrations, which generated four calibration curves for glycerin, monoolein, diolein, triolein, as reported in Figure 3. The linear correlation coefficients (r^2) exceeded the specification of 0.9 as requested in EN 14105 for each curve, demonstrating excellent linearity for this true cold On-column injection technique.

Sample Analyses

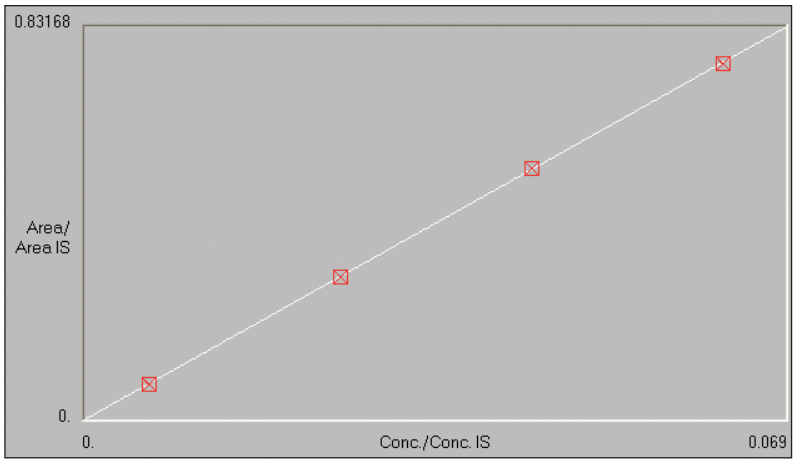
Following calibration, several biodiesel samples were then analyzed. Using the approach detailed in EN 14105 method, the amount of glycerin in each sample was calculated with the calibration function derived from the glycerin calibration curve. In the same way, the amount of mono-glycerides, di-glycerides and tri-glycerides were determined from the monoolein, diolein, and triolein calibration functions respectively. Peak identification for each group of compounds can be made using the relative retention times published in the EN 14105 method, using the retention time of IS1 as a reference for glycerol, and the retention time of IS2 as a reference for mono-, di-, and tri-glycerides groups. However, for this application, peak identification is based on comparison with the known standard components.

Figure 4a shows a typical biodiesel chromatogram, overlaid with the oven temperature program. Figure 4b displays the chromatogram for a calibration mixture. Figure 4c depicts the mono-glycerides check solution, which is analyzed to evaluate the exact position of the mono-glycerides in the unknown samples and to set the correct group window. For each group, the calculation of all the peak areas in every specified retention time window was automatically achieved using the Thermo Scientific Chrom-Card data system.

To highlight chromatographic performance and separation capabilities, Figure 5 offers details for each group of compounds from the biodiesel sample chromatogram in Figure 4.

Carryover Evaluation

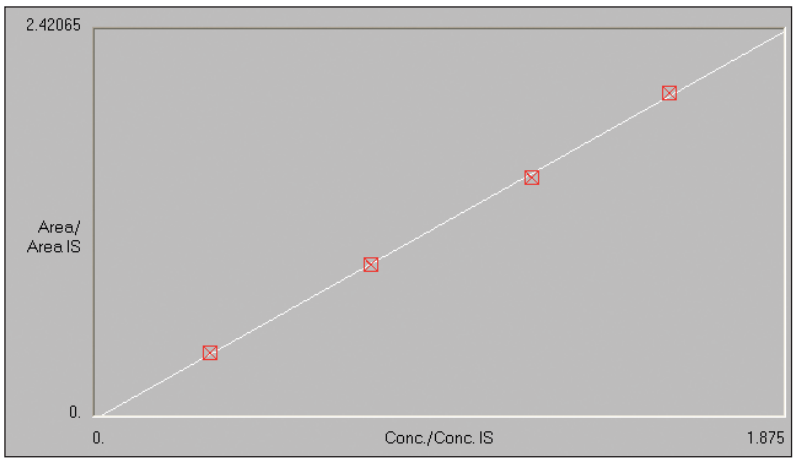
To evaluate the true On-column injection system for carryover, a blank chromatogram was obtained by injecting solvent only (Figure 6). Because this analysis was performed after a sequence of real biodiesel samples, it clearly demonstrates total absence of any carryover effect. Additionally, this evaluation shows that the heavy fraction completely elutes from the column during the previous analysis.



Glycerol

y = 12.08332 x + 0.000961

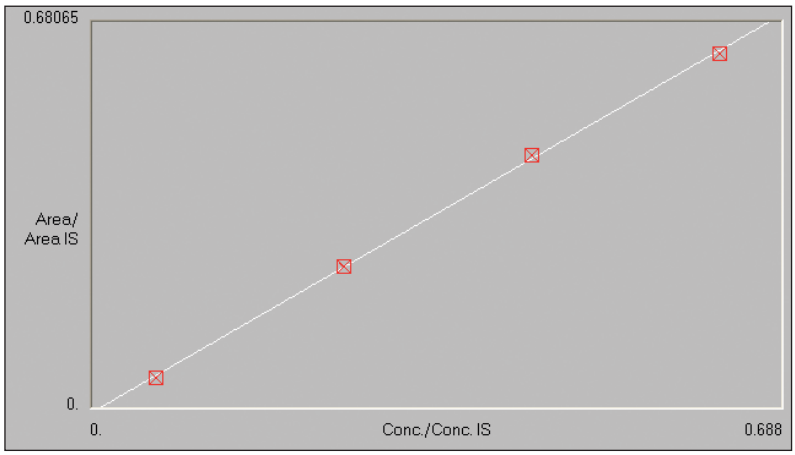
r^2 = 0.9999



Monoolein

y = 1.29527 x – 0.01902008

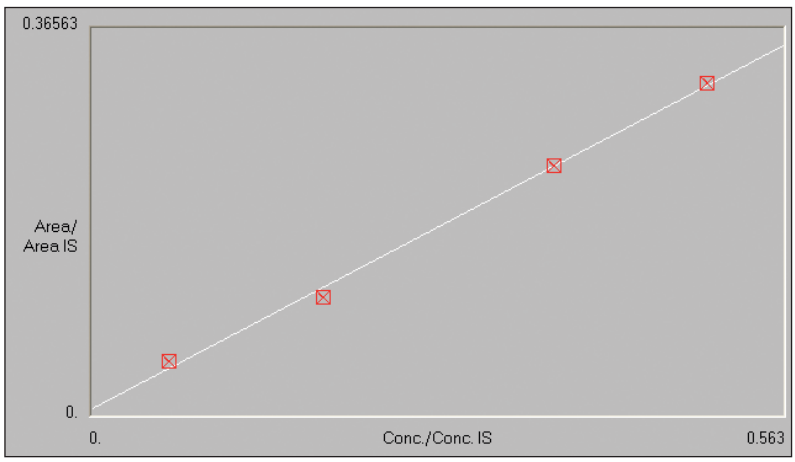
r^2 = 0.9992



Diolein

y = 1.020938 x – 0.007206254

r^2 = 0.9995



Triolein

y = 0.6099809 x + 0.006800001

r^2 = 0.997

Figure 3: Calibration curves for glycerol, monoolein, diolein, and triolein

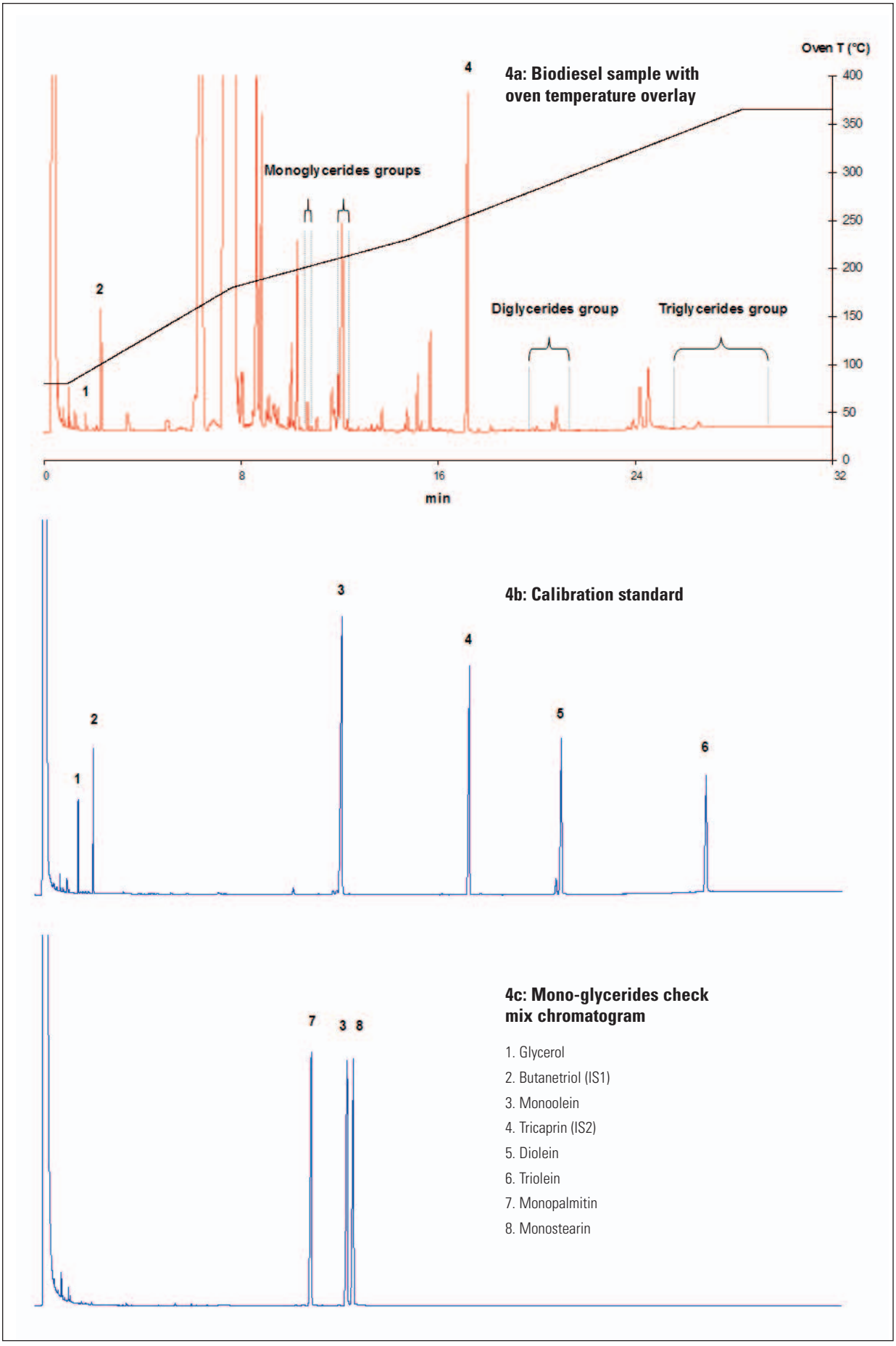


Figure 4: Biodiesel sample, standard calibration mix and mono-glycerides check mix chromatograms

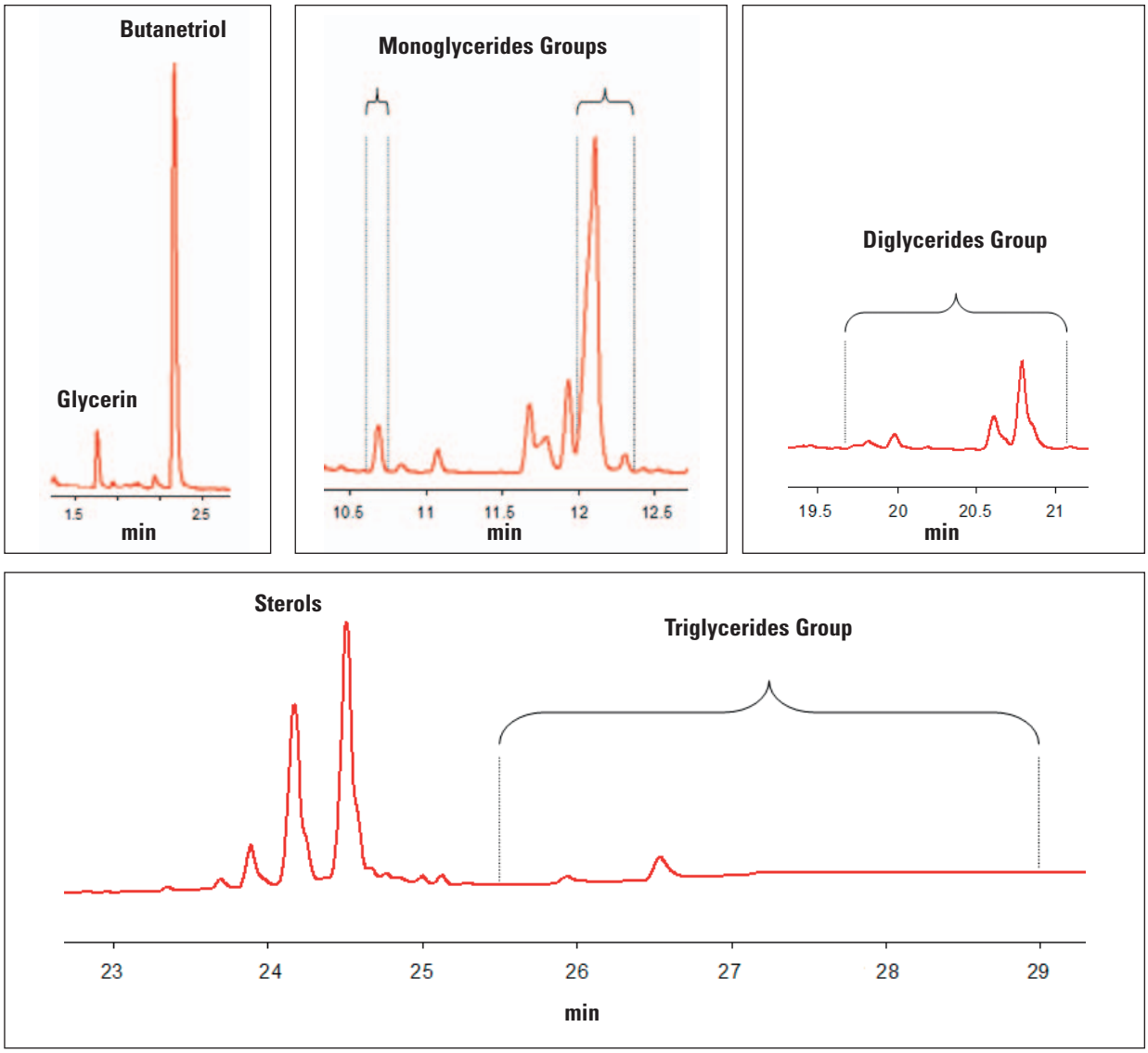


Figure 5: Details of the windows defined for the different groups of compounds in the biodiesel chromatogram

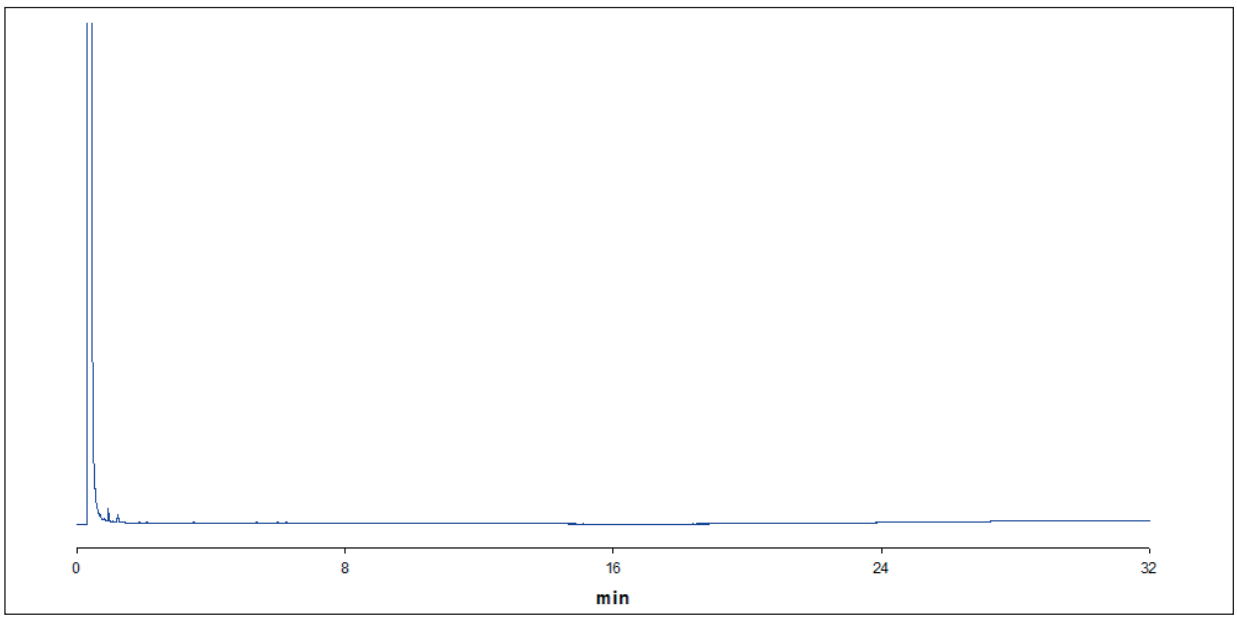


Figure 6: Blank chromatogram obtained after a sequence of real biodiesel samples

Repeatability Evaluation

Repeatability was calculated as 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 in EN 14105). Table 3 reports the results obtained for one of the analyzed biodiesel samples. In every instance, the se results exceeded the minimum performance requested by EN 14105, as indicated in the table. Similar results were obtained by repeating the double runs in different successive days.

	% m/m (average of 2 runs)	Experimental Repeatability (absolute %m/m)	Repeatability Limits - EN 14105 (absolute %m/m)
Free Glycerin	0.007	+ 0.0009	± 0.0018
Mono-glycerides	0.496	- 0.011	± 0.063
Di-glycerides	0.088	- 0.005	± 0.0093
Tri-glycerides	0.050	- 0.0070	± 0.0118
Total Glycerin	0.151	- 0.0030	± 0.0144

Table 3: Results of a biodiesel sample analysis

Conclusion

The determination of free and total glycerin in pure biodiesel (B100) can be achieved using the Thermo Scientific TRACE GC Ultra equipped with a true cold On-column inlet and FID, automated by the TriPlus liquid autosampler, in full compliance with the linearity and precision requirements of method EN 14105.

The true cold On-column injector has proven to prevent discrimination of the heavier fraction and to eliminate any risk of degradation of labile components like triglycerides, granting excellent recovery and absolute sample integrity. The specific TR-BIODIESEL(G) column can deliver enhanced mechanical robustness for this high oven temperature method, resulting in extended lifetime. The use of glass press-fit unions between the guard column and the analytical column can be avoided by using a low dead volume gas-purged metal connector, which ensures a continuous leak-free connection even when undergoing extremely large and frequent oven temperature variations. The use of an uncoated guard column is important to avoid any flooding effect and to prevent column contamination.

References

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