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Application News

Gas Chromatography

Determination of Free and Total Glycerol and Mono-, Di-, and Triglyceride Contents in Biodiesel According to EN 14105

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User Benefits

- Nexis™ GC-2060 offers reliable analysis of biodiesel samples regarding their free and total glycerol and mono-, di-, and triglyceride contents, using hydrogen as a cost-effective carrier gas
- Hydrogen carrier gas can be used safely through built-in safety measures
- The multi-mode injection unit (MMI) allows selection of the preferred injection mode – direct (simple OCI) or convenient splitless PTV mode – both delivering excellent instrument performance in line with the standard's requirements

■ Introduction

Playing a vital part in countries' efforts to lower greenhouse gas emissions, biofuels can be manufactured from various feedstocks such as vegetable oils, animal-derived fats, and recycled cooking oils. Biodiesel production predominantly uses rapeseed, soybean, and palm oils. Because feedstocks are so varied, ongoing quality monitoring is essential - since the raw material's quality greatly influences the final biodiesel quality.

The EN 14105:2024 describes the determination of free and total glycerol contents and mono-, di-, and triglyceride contents in biodiesel by means of gas chromatography with flame ionization detection (GC-FID). Injection shall be performed via on-column injector or an equivalent device[1].

This study shows the analysis of biodiesel originating from rapeseed, soybean, and palm oil using the Nexis GC-2060 gas chromatograph and its multi-mode injection unit (MMI).

■ Sample Preparation and Calculations

Sample preparation was performed according to EN 14105:2024. Glycerol and the mono- and diglycerides were freshly derivatized with MSTFA. 1 µL of the prepared sample solution was analyzed via GC-FID.

The free glycerol was quantified by means of an internal standard calibration via a series of calibration standards with 1,2,4-butanetriol as internal standard (ISTD). The glycerides were directly quantified in the sample using the respective ISTD compound per glyceride species: 1-glyceryl monononadecanoate (Mono C19), 1,3 glyceryl dinonadecanoate (Di C38), and glyceryl trinonadecanoate (Tri C57).

The total glycerol content was calculated from the above values with below formula:

$$G_{total} = G + 0.255\,M + 0.146\,D + 0.103\,T$$

with

G_{total} : Total glycerol content (in wt%)
G : Free glycerol content (in wt%)
M : Monoglyceride content (in wt%)
D : Diglyceride content (in wt%)
T : Triglyceride content (in wt%)

Instrument performance was monitored by evaluation of the relative response factor (RF) for Di C38 and Tri C57 using below formula:

$$RF = \frac{A_{DiC38}}{M_{DiC38}} \times \frac{M_{TriC57}}{A_{TriC57}}$$

with

A_{DiC38} : Area of Di C38 ISTD
M_{DiC38} : Weight of Di C38 ISTD
M_{TriC57} : Weight of Tri C57 ISTD
A_{TriC57} : Area of Tri C57 ISTD

The resulting RF value shall be below 1.8.

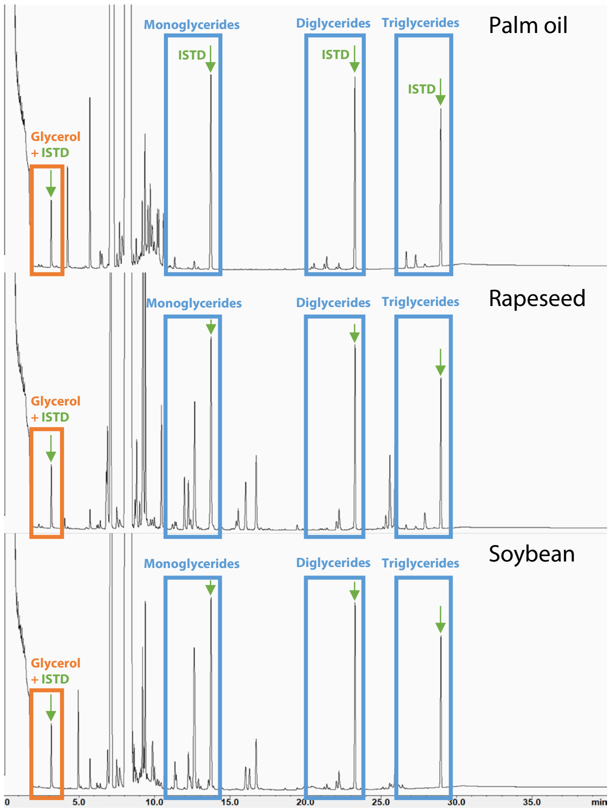

Fig. 1: Chromatograms of biodiesel samples (PTV mode): palm oil-based (top), rapeseed-based (middle), and soybean-based (bottom)

■ Results

EN 14105:2024 specifies that on-column injection or equivalent shall be used. The Shimadzu multi-mode injection unit (MMI) provides two alternative injection options for this purpose, which can be selected according to user preference: direct (simple OCI) and splitless programmed temperature vaporization mode (PTV). The mode is conveniently implemented by selecting the corresponding liner and setting the appropriate injection mode in the software. Exemplary biodiesel samples derived from rapeseed, soybean, and palm oil were analyzed in direct and PTV mode. Due to limited derivatization stability, the samples were freshly prepared for each injection mode (duplicate weighings per sample).

Figure 1 shows exemplary chromatograms of the three biodiesel samples obtained in PTV mode, highlighting the areas of interest for the free glycerol plus ISTD (orange) and the glycerides (blue). Figure 2 provides a direct comparison of a soybean-based biodiesel sample measured in direct mode (top) vs. PTV mode (bottom). The chromatograms are highly comparable and the calculated RF values are identical (1.2).

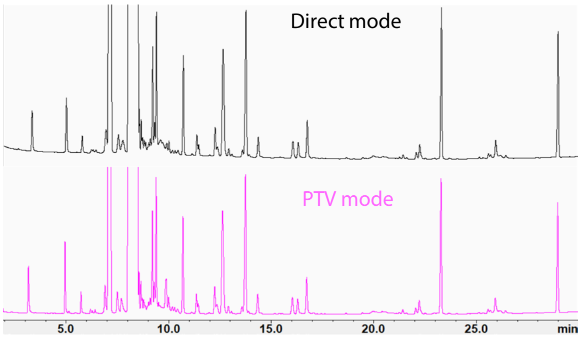

Fig. 2 Chromatograms of a soybean sample obtained in direct (top) and PTV mode (bottom) (zoomed to the regions of interest for better visibility)

In addition, the RF values monitored throughout the entire measurement series remained constant at 1.2 for all injections in both MMI modes. Together, these results demonstrate that PTV mode is a strong alternative to direct mode, as it does not increase triglyceride discrimination in the inlet, but maintains the good recovery of high-boiling compounds.
The EN 14105:2024 sets quantification limits of 0.10 % for the glycerides and 0.001 % for the free glycerol. Figure 3 shows the glycerol and ISTD peaks as well as the calibration curve derived from the calibration standards measured via PTV mode. Table 1 summarizes the glycerol and glyceride contents determined for the biodiesel samples in direct mode (left column) and PTV mode (right column). The results are highly comparable, further demonstrating PTV mode as a convenient, yet reliable alternative to direct mode. For the soybean sample, all target groups are above the quantification limit. In the rapeseed sample, the triglyceride content is at the quantification limit, while the palm oil sample has a monoglyceride content below the limit. All samples are below the maximum limits allowed for glycerol and glyceride contents in biodiesel set in the EN 14214:2019: 0.02 % for free glycerol, 0.25 % for total glycerol, 0.70 % for monoglycerides, and 0.20 % for diglycerides and triglycerides[2].

Table 1 Glycerol and glyceride contents (in %) of exemplary biodiesel samples measured in direct (left column) and PTV mode (right column)

Biodiesel	**Rapeseed**		**Palm oil**		**Soybean**	
MMI Mode	Direct	PTV	Direct	PTV	Direct	PTV
Monoglycer.	0.42	0.42	<0.1	<0.1	0.65	0.64
Diglycer.	0.12	0.12	0.10	0.10	0.13	0.12
Triglycer.	0.10	0.10	0.14	0.15	0.02	0.02
Free Glycerol	0.008	0.007	0.005	0.005	0.003	0.003
Tot. Glycerol	0.142	0.142	0.049	0.050	0.188	0.186

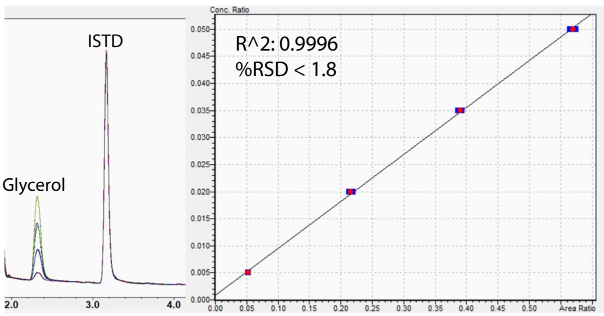

Fig. 3 Glycerol and ISTD peaks (left) and calibration curve (right) derived from the calibration standards measured in PTV mode

Fig. 4 Gas chromatograph Nexis™ GC-2060 with multi-mode injection unit (MMI), flame-ionization detector (FID) and autosampler AOC™-30i

■ Configuration and Analysis Conditions

Table 2 summarizes the instrument configuration (Fig. 4) and the analysis conditions used in this study.

Table 2 Instrument configuration and analysis conditions

Model	:	Nexis GC-2060 with MMI and AOC-30i
Column	:	SH-I-5HT (15m x 0.32 mm x 0.1 µm)*1
Carrier	:	80 kPa, hydrogen
MMI Mode	:	Direct or splitless PTV*2
MMI Temp.	:	50 °C – 250 °C/min – 370 °C
Column Temp.	:	50 °C (0.5 min) – 15 °C/min – 180 °C – 7 °C/min – 230 °C – 10 °C/min – 370 °C (9.7 min)
FID	:	380 °C, default gas flows

*1 P/N: 227-36305-02, *2 P/N: 227-36703-01 (direct) or 227-36701-01 (PTV)

■ Conclusion

Nexis GC-2060 provides reliable analysis of biodiesel samples for glycerol and glyceride contents according to EN 14105:2024 across the commonly used feedstocks. It supports hydrogen as a cost-effective carrier gas, while the multi-mode injection unit (MMI) offers selection of the injection mode based on user preference: direct mode representing simple on-column (OCI) analysis or splitless programmed temperature vaporization (PTV) mode as a convenient alternative that delivers comparable performance.

<Acknowledgments>

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<References>

1) EN 14105:2024-14, - Fat and oil derivatives - Fatty Acid Methyl Esters (FAME) - Determination of free and total glycerol and mono-, di-, triglyceride content
2) EN 14214:2019-05, - Liquid petroleum products - Fatty acid methyl esters (FAME) for use in diesel engines and heating applications - Requirements and test methods

<Related Applications>

1. Determination of Ester and Linolenic Acid Methyl Ester Contents in Biodiesel According to EN 14103:2020 Application News No. 05-SCA-180-054A-EN

05-SCA-180-055-EN First Edition: Aug. 2026

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