

Application Note # CA-270357

Determination of Free and Total Glycerine and mono-, di-, triglyceride content in B-100 Biodiesel Methyl Esters by Gas Chromatography

Introduction

The European Standard method, EN-14105, is used to determine the free glycerol and residual mono-, di- and triglyceride contents in fatty acid methyl esters (FAME) typically intended for addition to mineral oils. Total glycerol content is calculated from the results obtained. The method is suitable for FAME from rapeseed, sunflower and soybean oils, but is not suitable for FAME produced from or containing coconut and palmkernel oils due to the problem of peak overlapping. This method and ASTM D6584 are two of the most commonly used standardized analytical methods used for the analysis of biodiesel.

Instrumentation

Bruker 450 Gas Chromatograph

- Injector: Cold on-column (1093), full EFC control
- Detector: FID, full EFC control
- Automatic Liquid Sampler: CP-8410 (or CP-8400)

GC control and data handling software

- Galaxie™ GC Workstation

Materials and Reagents

- Column: Select™ Biodiesel for Glycerides 10 m x 0.32 mm (id) x 0.1 µm (film) (part no: CP-9076)
- Retention gap: 2.5 m x 0.53 (id)
- Connector: stainless steel ZDV (zero dead volume)
- Carrier gas (Helium or Hydrogen; 99.999 pure or better)
- N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA)
- N-Heptane
- Pyridine
- 1,2,4-Butanetriol (Internal Standard Solution 1)
- 1,2,3-Tricaproylglycerol (Internal Standard Solution 2)
- Reference materials: glycerol, 1=monooleoylglycerol, 1,3-dioleoylglycerol (GLC standard grade)
- Volumetric flasks 50 and 100 mL
- Glass micro-syringes; 1, 100 and 500 µL capacity
- Precision pipette (1 mL)
- 10 mL glass vials and caps with PTFE faced septa

Sample Preparation

Calibration mixtures and internal standard solutions were prepared according to the method. 100 mg of homogenized sample was accurately weighed in a 10 mL vial. Then, using a glass syringe, the following was added to the vial: 10 µL of internal standard No. 1 stock solution, 100 µL of internal standard No. 2 stock solution and 100 µL of MSTFA. Care was taken to ensure there was no contact with moisture. The vials were hermetically sealed and shaken vigorously. After storing the vials at room temperature for ~ 15 minutes, 8 mL of heptane was added to each. Then, 1 µL of the reaction mixture was automatically injected into the gas chromatograph according to the instrumental conditions described below (see Table 1 for typical results).

Conditions

Injection: Cool, on-column, 1 µL (after injection, the injector follows the temperature program of the oven)

Detector: FID, 380 °C

Oven: 50 °C, (1 min) @ 15 °C/min to 180 °C

@ 7 °C/min to 230 °C

@ 10 °C/min to 370 °C (5 min)

Carrier Gas: Helium, 80 kPa

Results and Discussion

The method describes the transformation (via derivatization) of the glycerol and of the mono- and diglycerides into volatile silylated derivatives in presence of pyridine and N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA).

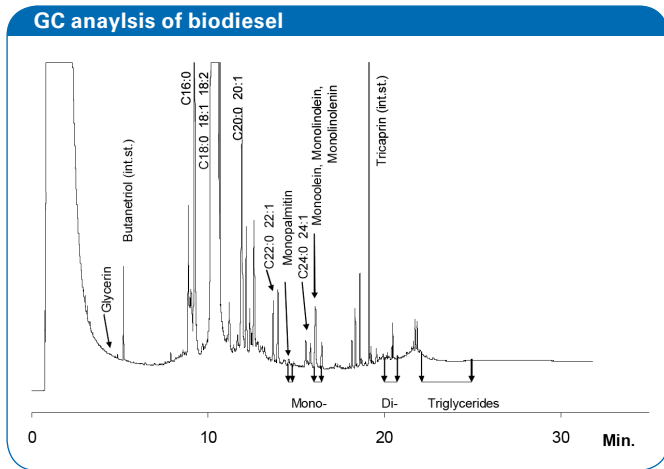


Figure 1: Biodiesel chromatogram.

Conclusion

This application note demonstrates the suitability of Bruker's 450-GC equipped with a cold on-column injector and FactorFour™ high temperature column for the analysis of biodiesel. The calibration curves and repeatability data demonstrate excellent system integrity. Making the system well suited for the analysis of free and total glycerol and mono, di, and triglyceride content in biodiesel in accordance with the standard method EN-14105.

Table 1: Typical analysis results of a biodiesel (B-100) sample.

File	Glycerine (mass %)	Monoglycerides (mass %)	Diglycerides (mass %)	Triglycerides (mass %)	Totals
2_1	0.00975	0.69191	0.06116	0.06234	0.82515
2_2	0.00976	0.71111	0.06304	0.06063	0.84455
2_3	0.00998	0.68955	0.06491	0.06175	0.82618
2_4	0.00977	0.67079	0.06429	0.06165	0.80650
2_5	0.00984	0.67629	0.06290	0.05548	0.80451
2_6	0.00985	0.68240	0.06629	0.05770	0.81624
2_7	0.00980	0.69243	0.06236	0.06057	0.82516
2_8	0.01012	0.60468	0.06489	0.06096	0.74065
2_9	0.00982	0.73039	0.06448	0.06212	0.86680
2_10	0.01014	0.72353	0.06282	0.05948	0.85597
Average	0.009883	0.687308	0.063714	0.060268	0.82117
St.Dev.	0.00015	0.03501	0.00151	0.00218	0.03480
St.Dev. (%)	1.5	5.1	2.4	3.6	4.2

Calibration curves are obtained for Glycerine, Monoolein, Diolein and Triolein. These calibration curves give an indication on the performance of the system. Typical calibration curves for Glycerine and Diolein are shown in Figures 2 and 3.

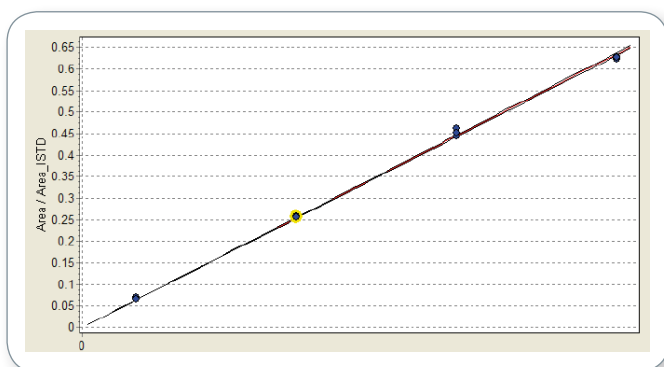


Figure 2: Calibration curve: Biodiesel 14105 2nd calibration - Glycerine.

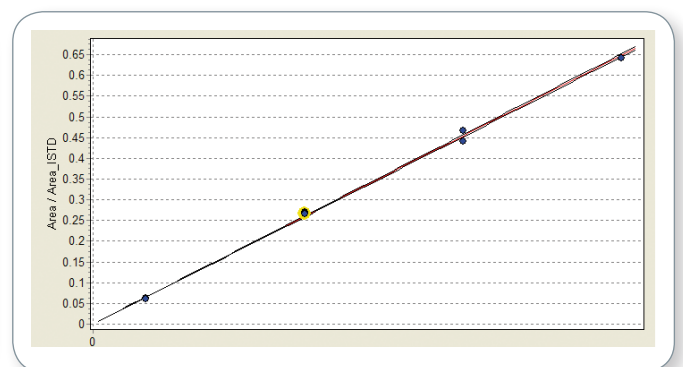


Figure 3: Calibration curve: Biodiesel 14105 2nd calibration - Diolein.

Table 2: Typical analysis results of a biodiesel (B-100) sample.

Index	Name	Area (µV.Min)	Quantity (mass %)
1	Glycerine	199.8	0.00975
2	Monoglycerides	5692.9	0.69191
3	Diglycerides	758.0	0.06116
4	Triglycerides	714.6	0.06234
Total		7365.3	0.82515

Keywords
Biodiesel
Glycerol
mono, di, and triglyceride
EN-14105

Instrumentation & Software
Bruker 450
Gas Chromatograph
Galaxie™ GC Workstation

For research use only. Not for use in diagnostic procedures.

References

- [1] Fat and oil derivatives – Fatty Acid Methyl Esters (FAME) – Determination of free and total glycerol and mono-, di-, triglyceride content (Reference method); EN-14105 (European Committee for Standardisation; Management Centre, rue de Stassart 36, B-1050 Brussels, Belgium).