APPLICATION NOTE

Gas Chromatography

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Fatty Acid Methyl Esters in B100 Biodiesel by Gas Chromatography (Modified EN 14103)

Introduction

The production and consumption of biofuels continues to increase as more attention is paid to the environment and the depletion of fossil-fuel resources. Biodiesel, a fuel from natural oils such as soybean oil, rapeseed oil or animal fats, is a substitute for petroleum-diesel fuel. The quality criteria for the production of biodiesel are specified in EN 14214.

Within EN 14214, method EN 14103 specifies the fatty acid methyl ester (FAME) and linolenic acid methyl ester content (Figure 1), which is used to profile the vegetable or animal oil feedstock used in biodiesel production. EN 14103 calls for calibration of all FAME components by relative response to a single compound, methyl heptadecanoate. This requires the measurement of accurate weights for each sample and the addition of an internal standard. The range of FAMEs for which the method is intended lies between $C_{14:0}$ and $C_{24:1}$.

This application note will discuss the analysis according to method EN 14103. In addition to the methodology specified in EN 14103, a simpler and more accurate method will be presented. The modified method uses commercially-available calibration and test mixtures for precise peak identification and quantitative accuracy, while streamlining the sample preparation and calculations. Reporting is based on area % of all components after the solvent – as a result, the sample weight does not impact the calculations.

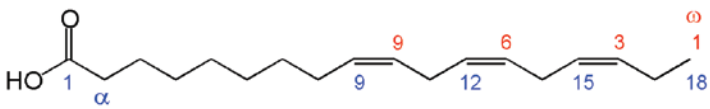

Figure 1. Linolenic acid.

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Experimental

The FAME analysis is carried out with a split injection onto an analytical column with a polar stationary phase and an FID detector. The configuration used here is the PerkinElmer® Clarus® Gas Chromatograph (GC), fitted with a capillary split/splitless injector and FID. It is very important to choose the appropriate liner; otherwise, the response, reproducibility and resolution of the analysis will be compromised. Fatty acid methyl esters are known to be active and thermally-labile components. Incorrect injection conditions and liner choice will result in a non-linear response; the late-eluting FAMEs (longer carbon chains) show a lower response than the early-eluting FAMEs. This problem is overcome by packing the inlet liner with glass wool, greatly improving uniformity and reproducibility over the boiling-point range. Deactivated liners (with wool) are available. The surface of the liner is deactivated to minimize bleed and to enhance the inertness of the liner.

The analytical column used in this work is the PerkinElmer Elite-Famewax column (Crossbond® polyethylene glycol), which demonstrates good resolution and peak shape (Figure 2). Table 1 provides an overview of all the instrument parameters.

In order to determine the retention times of the fatty acid methyl esters, a FAME standard needs to be run. These are available commercially either separately or as a standard reference mixture. The analysis of a commercial FAME standard is shown in Figure 2.

Table 1. **Instrument Parameters EN 14103.**

Gas Chromatograph:	PerkinElmer Clarus GC
Inlet Temperature:	250 °C
Column Flow:	1 mL/min
Split Flow:	50 mL/min
Injection Volume:	0.5 µL
Oven Program Initial Temp: Hold Time 1: Ramp 1:	210 °C 13.00 min 5 °C/min
Oven Program Final Temp: Hold Time 2:	230 °C 15.00 min
Equilibration Time:	0.0 min
Column:	Elite-Famewax 30 m x 320 µm x 0.25 µm film
Carrier Gas:	Helium
FID Temperature: H_2 Flow: Air Flow: Range: Attenuation:	250 °C 45 mL/min 450 mL/min 1 -5

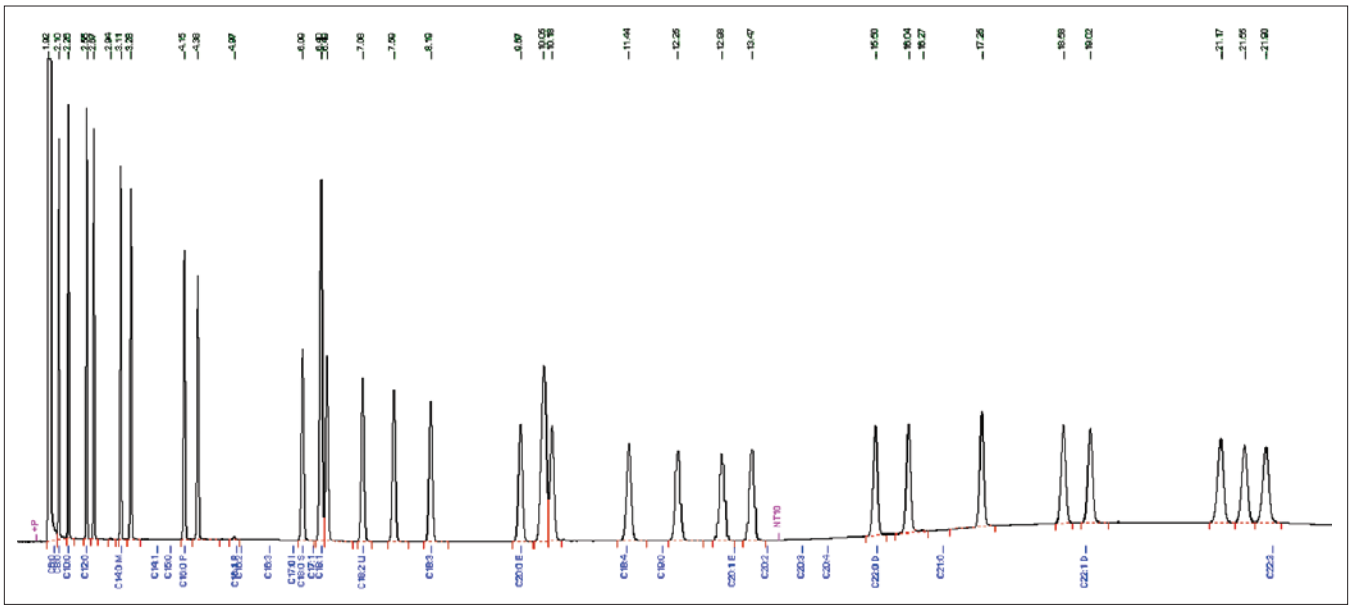

Figure 2. The analysis of a mixture of $C_{14:0}$ - $C_{24:1}$ FAMEs.

The modified method of analysis uses a FAME mixture at known concentration and determines the response and retention time of each component experimentally. The reporting is then based on an area % rather than a mass %, simplifying the calculations. The calibration is verified by the analysis of a calibration-check standard – typically, this is commercial mix of FAMEs from a second source with a certificate of analysis. This standard would be analyzed after the calibration of the response. The results of the analysis are then compared with the certificate of analysis, verifying the quality of the calibration. The standard preparation for this technique consists of the dilution of the FAME standard into 4 mL of n-heptane. The sample preparation is also quite simple with 100 µL of biodiesel feedstock into 4 mL of n-heptane. In both cases, a 1-µL injection at a split of 50:1 is performed. The full instrument conditions are presented in Table 2.

***Table 2.* Modified Parameters for the Analysis of FAMEs in Biodiesel.**

Gas Chromatograph:	PerkinElmer Clarus GC
Inlet Temperature:	240 °C
Column Flow:	2 mL/min
Split Flow:	50 mL/min
Injection Volume:	1 µL
Oven Program Initial Temp: Hold Time 1: Ramp 1:	195 °C 0 min 5 °C/min
Oven Program Final Temp: Hold Time 2:	240 °C 6 min
Column:	Carbowax 20 M 30 m x 320 µm x 0.25 µm film
Carrier Gas:	Helium
FID Temperature: H_2 Flow:	240 °C 45 mL/min
Air Flow:	450 mL/min

Results

In EN 14103, the result for the fatty acid methyl ester content is expressed as a mass fraction in percent using methyl heptadecanoate (C_{17}) as the internal standard. Total FAME content should be greater than 90%. Linolenic acid ($C_{18:3}$) content should be greater than 1% and less than 15%. The following formula is used:

$$C = \frac{\Sigma A - A_{IS}}{A_{IS}} \times \frac{C_{IS} \times V_{IS}}{m} \times 100\%$$

Where:

SA = total peak area $C_{14:0}$ – $C_{24:1}$

AIS = internal standard (methyl heptadecanoate) peak area

CIS = concentration of the internal standard solution, in mg/mL

VIS = volume of the internal standard solution used, mL

m = mass of the sample, in mg

Linolenic acid methyl ester content is also expressed as a mass fraction in percent and methyl heptadecanoate (C_{17}) is used as the internal standard. The following formula applies:

$$L = \frac{A_L}{\Sigma A - A_{IS}} \times 100\%$$

Where:

SA = total peak area $C_{14:0}$ – $C_{24:1}$

AIS = internal standard (methyl heptadecanoate) peak area

AL = linolenic acid methyl ester peak area

The result of the mass fraction calculation is then used to calculate the sample's iodine value, which is the sum of the individual contributions of each methyl ester, obtained by multiplying the methyl ester percentage by its respective factor. The following formula applies:

Iodine value = X g iodine / 100 g sample

Table 3 shows an example calculation for the iodine factor.

***Table 3.* Iodine Factor Example.**

Methyl Ester of Following Acids in Sample (as example)	Amount in % Mass	Iodine Factor	Contribution
Myristic $C_{14:0}$	0.3	0	0.00
Palmitic $C_{16:0}$	4.0	0	0.00
Palmitoleic $C_{16:1}$	1.1	0.950	1.05
Stearic $C_{18:0}$	2.0	0	0.00
Oleic $C_{18:1}$	60.5	0.860	52.03
Linoleic $C_{18:2}$	19.8	1.732	34.29
Linolenic $C_{18:3}$	9.4	2.616	24.59
Eicosanoic $C_{20:0}$	0.4	0	0.00
Eicosenoic $C_{20:1}$	0.7	0.785	0.55
Docosanoic $C_{22:0}$	0.7	0	0.00
Docosenoic $C_{22:1}$	1.1	0.723	0.80
		Calculated Iodine Value	**113.3**

Conclusion

Method EN 14103 is used to determine the fatty acid methyl ester (FAME) between $C_{14:0}$ and $C_{24:1}$ and linolenic acid methyl ester content of oil feedstock used in biodiesel production. EN 14103 calls for calibration of all FAME components by relative response to a single compound – methyl heptadecanoate. This application note has demonstrated the analysis according to method EN 14103. Additional methodology presented a simple analysis using commercially-available calibration and test mixtures.

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