EN 15779 - Gas Chromatographic Analysis of Polyunsaturated FAME in Biodiesel Made From Algae and Marine Oils

Application Note

Fuels

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Abstract

The GC analysis of four common polyunsaturated fatty acid methyl esters (PUFA FAMEs) in algal biodiesel is described using method EN 15779. An Agilent 7890A GC system was configured and calibrated according to the procedure outlined in the method. Two samples of B100 biodiesel made from algae oil were each prepared in duplicate and analyzed according to the conditions set forth in the method. In each sample, the four PUFA FAMEs were chromatographically separated and quantified. The analysis precision was calculated and shown to exceed the specifications of the EN 15779 methods.

Introduction

Currently, most worldwide stocks of biodiesel are made from vegetable oils or animal fats. While these sources are cheap and convenient, they compete with food production resources. Current research involves finding nonfood sources of triglycerides harvested from plants that do not compete with food production. A promising source is algae cultivated in contained bioreactors, where both growth rates and oil yields are greater when compared to land-based crops. One potential problem with algae and marine oils is the high concentrations of polyunsaturated fatty acids (PUFA). After conversion to biodiesel fuel, PUFA FAMEs exhibit lower oxidation stability and higher rates of self-polymerization. These properties can cause engine fouling and fuel line or filter plugging if the PUFA FAME content is too high.

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To assure good algal biodiesel quality, the European Committee for Standardization (CEN) has developed a GC method to measure the amount of four predominant PUFA FAMEs found in these biodiesels (Table 1). The method is designated as EN 15779 [1]. This application note describes the configuration and performance of the Agilent 7890A GC system when using this method for the analysis of B100 biodiesel derived from algae oil.

Table 1. Polyunsaturated FAMEs Measured Using Method EN 15779

CAS number	Chemical name	Abbreviation
2566-89-4	Methyl eicosatetraenoate	C20:4 (n-6)
2734-47-6	Methyl eicosapentaenoate	C20:5 (n-3)
108698-02-8	Methyl docosapentaenoate	C22:5 (n-3)
28061-46-3	Methyl docosahexaenoate	C22:6 (n-3)

Experimental

An Agilent 7890A GC was configured and the instrument conditions were set according to the EN 15779 method. These details are shown in Tables 2 and 3. A 1.0 mg/mL solution of methyl tricosanoate (C23:0) in n-heptane was prepared for use as an internal standard. A 0.1 mg/mL solution of the four PUFA FAMEs (Table 1) was prepared in n-heptane containing 1.0 mg/mL of the internal standard (C23:0). This standard was used to determine the retention times for each PUFA FAME and the C23:0 internal standard. Two samples of algal B100 biodiesel were obtained for testing. Each sample was prepared by weighing 100 mg into a 2-mL autosampler vial and adding 1.0 mL of the C23:0 internal standard solution followed by mixing. The samples were prepared and run in duplicate to determine the repeatability of the analysis.

Table 2. 7890A GC Configuration for EN 15779

Standard Agilent 7890A GC system hardware	
Agilent 7890A Series GC (G3440A)	
Option 112	100 psi split/splitless Inlet with EPC control
Option 211	Capillary FID with EPC control
Agilent 7693 Autoinjector (G4513A)	
123-7032	DB-Wax Column, 0.32 mm × 30 m id × 0.25 µm

Table 3. Instrument Conditions for EN 15779 Method

Column oven conditions	
Initial oven temperature	150 °C for 1 min
Oven ramp 1	15 °C/min to 200 °C
Oven ramp 2	2 °C/min to 250 °C
Inlet and sampling conditions	
Column flow	Hydrogen at 1 mL/min constant flow
Inlet temperature	220 °C
Inlet mode	Split at 50:1 split ratio
Injection size	1 µL
Flame ionization detector conditions	
Detector temperature	250 °C

Results and Discussion

Figure 1 shows a chromatogram of the PUFA FAME reference standard run under the EN 15779 GC conditions. The retention times of each peak were noted on the chromatogram. These retention times were used to identify each of the four PUFA FAMEs found in the biodiesel samples.

The GC analysis of the two algal biodiesel samples is shown in Figure 2. The FAME profiles of the two samples are very similar, but the PUFA FAME content appears higher in sample 1. Quantification of the PUFA FAMEs was done using the theoretical response factors for each PUFA FAME published in the EN 15779 method. These response factors were corrected using the detector response of the C23:0 FAME internal standard added to each sample. This procedure helps to improve the accuracy of the final results. The weight percent of each PUFA FAME was calculated, and the total PUFA FAME content in the samples was reported by summing the individual FAMEs. Table 4 shows the results for the duplicate analyses of both algal biodiesel samples.

The analysis precision for each sample was determined by calculating the repeatability (r) for the duplicate runs. Repeatability is defined as the difference between duplicate sample results analyzed by a single operator on the same equipment in a short period of time, usually the same day. For the EN 15779 method, a repeatability specification was only determined for the total PUFA FAME result. Table 4 shows that this specification was exceeded for both samples when using the Agilent 7890A GC system.

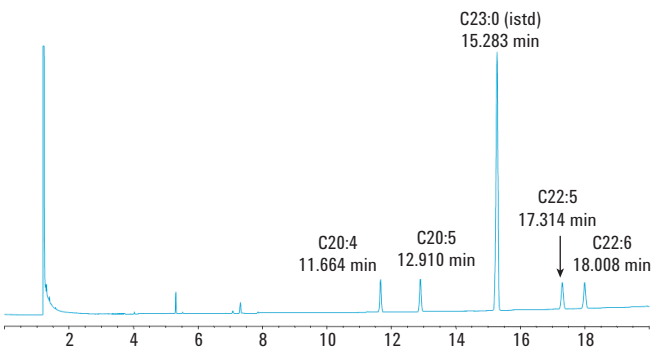

Figure 1. Chromatogram of the retention time standard containing the four PUFA FAMEs and the internal standard, methyl tricosonate (C23:0).

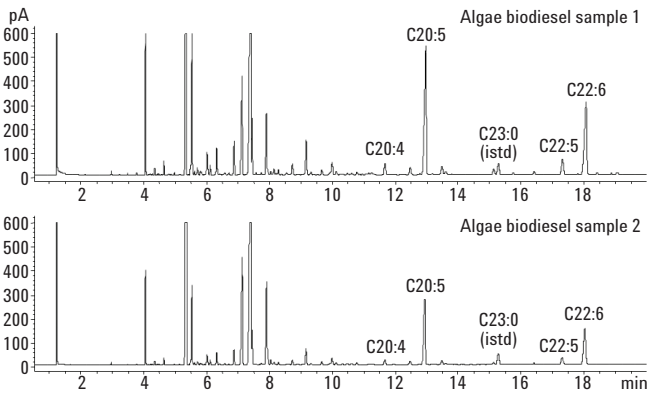

Figure 2. These chromatograms show the analysis of PUFA FAMEs in biodiesel samples made from two different algae oils.

Table 4. Reproducibility of Biodiesel Sample Runs

Run	C20:4 wt%	C20:5 wt%	C22:4 wt%	C22:6 wt%	Total PUFA
Algae biodiesel sample 1					
1	0.39	5.96	0.72	4.01	11.08
2	0.39	5.98	0.72	4.02	11.11
			Measured repeatability (r)		0.03
			EN 15779 Specification (r)		0.07
Algae biodiesel sample 2					
1	0.17	2.65	0.32	1.79	4.93
2	0.18	2.67	0.32	1.81	4.98
			Measured repeatability (r)		0.05
			EN 15779 Specification (r)		0.07

Excellent precision was observed for duplicate runs of each algal biodiesel sample. The reproducibility (r) measured for each sample exceeded the specification published in the EN 15779 method.

Conclusion

The analysis of PUFA FAMEs in biodiesel made from algal or marine oils can be easily done using EN method 15779 on an Agilent 7890A GC system. Calibration and reporting of the PUFA FAME content can be done according to the method's protocol using the standard tools within the Agilent Chemstation. After analyzing two algal oil biodiesel samples, the 7890A GC system provided results whose precision met the requirement of the EN 15779 method.

References

1. "EN15779 Petroleum products and fat and oil derivatives – Fatty acid methyl esters (FAME) for diesel engines – Determination of polyunsaturated fatty acid methyl esters (PUFA) by gas chromatography"; European Committee for Standardization: Management Centre, Avenue Matrix 17, B-1000 Brussels, 2009.

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