



Analysis of FAMES in Biodiesel Fuel: Pro EZGC Modeling Software Ensures Proper Column Selection

By Katarina Oden

Abstract

Polar columns were evaluated for the analysis of fatty acids methyl esters (FAMES) in finished B100 biodiesel according to method EN 14103 (2011). Using Restek's Pro EZGC chromatogram modeler, a high cyano phase Rt-2330 column and a polyethylene glycol phase FAMEWAX column were compared. The modeling software predicted an unacceptable coelution between the internal standard (C19:0 FAME) and FAME C18:2 when using the Rt-2330 column. However, the modeler also predicted that the FAMEWAX column would separate all the compounds of interest, which was demonstrated empirically. In addition, the results on the FAMEWAX column showed excellent repeatability for both total FAMES and the linolenic acid methyl ester component.

Introduction

Biodiesel is a diesel fuel made from plant or animal fat feedstocks. These biologically sourced fats, predominantly triglycerides (1), are converted into fatty acid methyl esters (FAMES) via a transesterification reaction that occurs in the presence of methanol and a basic or acidic catalyst. This reaction produces biodiesel fuel and also generates glycerol as a byproduct. The biodiesel FAME profile is determined by the type of fat that is used in the reaction and, therefore, the specific composition of biodiesel can vary from saturated to unsaturated FAMES. The ester composition of biodiesel is used to determine product quality and to calculate its cetane number. According to the method EN 14214, which regulates biodiesel quality, ester content in 100% biodiesel (B100 product) has to be greater than 90% total fatty acid methyl esters by mass. In addition, the linolenic acid methyl ester (methyl linolenate) content must be between 1% and 15% by mass (2).

European standard method EN 14103 (2011) is widely used for the analysis of FAMES in biodiesel. It is specifically used to determine both the FAME composition and, simultaneously, the linolenic acid methyl ester concentration. Linolenic acid methyl ester is a methyl ester of a polyunsaturated fatty acid where both *trans* and *cis* isomers can be present. A high concentration of linolenic acid methyl ester is undesirable because its poor oxidation stability can change fuel properties and form undesirable species (3).

According to method EN 14103 (2011), polar FAMES in biodiesel can be resolved and quantitated using gas chromatography and highly polar capillary column (2). Polar columns, such as high cyano (Rt-2330) or polyethylene glycol columns (FAMEWAX) offer excellent retention and selectivity for polar FAME compounds. This application note uses the Pro EZGC chromatogram modeling software to assess the performance of two polar analytical columns for EN 14103 (2011) biodiesel analysis and then compares the model output to empirical data.

Experimental

Rt-2330 and FAMEWAX columns were selected for this experiment because they are highly polar phases that have been shown to generally perform well for FAMES analysis. Both columns were initially evaluated using two criteria: selectivity using Pro EZGC chromatogram modeling software and overall method suitability. The Pro EZGC modeler conditions were customized to match EN 14103 (2011) operating conditions and the most prevalent FAMES were chosen and modeled along with C19:0 as an internal standard.

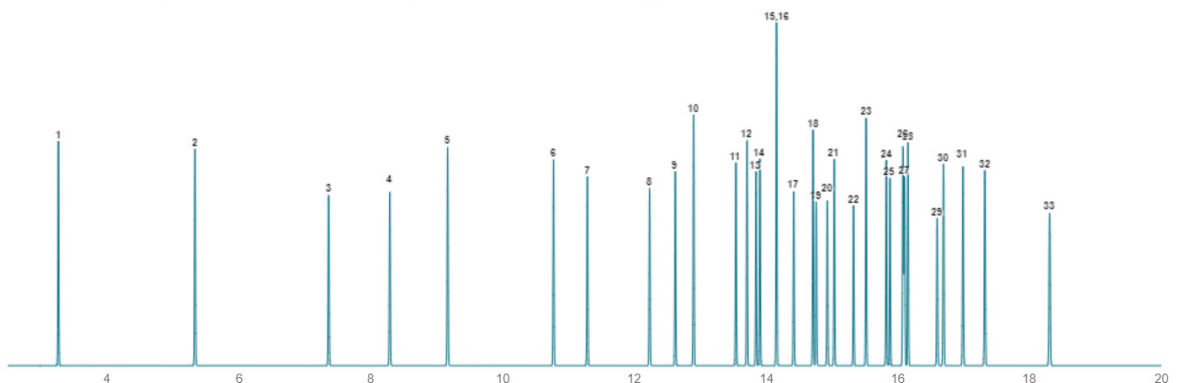
The results from the most promising modeled chromatogram were confirmed in the laboratory by analyzing a Restek food industry FAME standard (30 mg/mL in methylene chloride, cat.# 35077) and a single compound FAME C19:0 internal standard (10 mg/mL in toluene, cat.# 35055) on a FAMEWAX 30 m x 0.25 mm x 0.25 µm column (cat.# 12497). Commercially obtained canola and soy biodiesel B100 samples were also analyzed following method EN 14103 (2011).

In order to assess repeatability, total ester content and linolenic acid methyl ester content over multiple analyses were calculated as described in the method.

Results and Discussion

The modeled Pro EZGC chromatogram for the Rt-2330 column (Figure 1) clearly illustrates a coelution between the internal standard FAME C19:0 and *trans* C18:2 FAME, which can be present in biodiesel. This means that the Rt-2330 column is not suitable for the analysis of FAMEs in biodiesel under the method conditions because the internal standard was not completely resolved. Predicting this problem using the Pro EZGC chromatogram modeler took only minutes on the computer, providing a substantial savings of time and money compared to determining this experimentally in the lab.

Figure 1: Pro EZGC modeling software predicts the coelution of internal standard FAME C19:0 with *trans* C18:2 FAME on an Rt-2330 column. This allows analysts to remove the column from consideration without the time and expense of testing its performance in the lab.

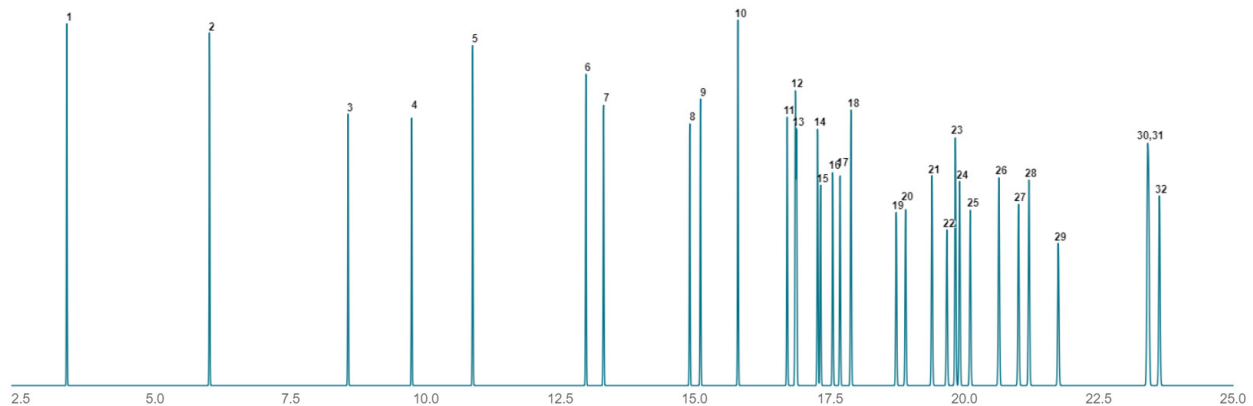


Peaks	t _r (min)	R _s	Peak Width (min)	T _{peak} (°C)	Peaks	t _r (min)	R _s	Peak Width (min)	T _{peak} (°C)
1. C6:0	3.25	73.9	0.028	72.5	18. C18:3 (all-cis-6,9,12)	14.71	1.5	0.030	187.1
2. C8:0	5.32	66.4	0.031	93.2	19. C20:0	14.76	1.5	0.029	187.6
3. C10:0	7.35	30.4	0.031	113.5	20. C18:3 (all-cis-9,12,15)	14.93	3.4	0.031	189.3
4. C11:0	8.28	28.8	0.031	122.8	21. C20:1 (cis-11)	15.03	3.4	0.030	190.3
5. C12:0	9.16	28.8	0.031	131.6	22. C21:0	15.32	6.5	0.029	193.3
6. C14:0	10.77	17.1	0.030	147.7	23. C20:2 (all-cis-11,14)	15.52	6.5	0.030	195.2
7. C14:1 (cis-9)	11.28	17.1	0.030	152.8	24. C20:3 (all-cis-8,11,14)	15.82	1.8	0.030	198.2
8. C16:0	12.23	13	0.030	162.3	25. C22:0	15.88	1.8	0.029	198.8
9. C16:1 (cis-9)	12.62	9.2	0.030	166.2	26. C20:3 (all-cis-11,14,17)	16.07	0.7	0.030	200.4
10. C17:0	12.89	9.2	0.030	168.9	27. C20:4 (all-cis-5,8,11,14)	16.10	0.7	0.031	200.5
11. C18:0	13.54	5.6	0.029	175.4	28. C22:1 (cis-13)	16.15	1.7	0.030	200.8
12. C18:1 (trans-9)	13.71	4.6	0.030	177.1	29. C22:2 (all-cis-13,16)	16.59	2.9	0.032	203.0
13. C18:1 (cis-9)	13.84	1.9	0.030	178.4	30. C20:5 (all-cis-5,8,11,14,17)	16.69	2.9	0.033	203.4
14. C18:1 (cis-11)	13.90	1.9	0.030	179.0	31. C24:0	16.99	8.8	0.033	204.9
15. C18:2 (all-trans-9,12)	14.15	--	0.030	181.5	32. C24:1 (cis-15)	17.32	10.2	0.034	206.6
16. C19:0	14.15	--	0.029	181.6	33. C22:6 (all-cis-4,7,10,13,16,19)	18.30	28.7	0.039	211.5
17. C18:2 (all-cis-9,12)	14.41	8.8	0.030	184.2					

Column: Rt®-2330, 30.00 m, 0.25 mm ID, 0.25 µm
 Carrier Gas: Hydrogen, Constant Flow @ 1.75 mL/min
 Average Velocity: 48.32 cm/sec
 Outlet Pressure (abs): 14.70 psi (Atmospheric Pressure)
 Oven Temp: 60 °C (hold 2 min) to 200 °C @ 10 °C/min to 240 °C @ 5 °C/min

The Pro EZGC chromatogram modeler is not only useful in determining that a column will not work, it also can be used to predict what column phase will work best for the analysis of FAMES in biodiesel according to method EN 14103 (2011). As shown in Figure 2, the modeled FAMEWAX chromatogram predicts that the critical separation of linolenic acid methyl ester will be achieved as well as complete resolution of the internal standard. While another coelution is predicted later in the chromatogram, the internal standard is completely resolved and the other coelution is not critical because the FAME peak areas will be summed according to the method. Based on the promising nature of the modeled output, chromatographic results were confirmed in the lab and the actual FAMEWAX column analysis sufficiently matched the predicted results. As shown in Figure 3, the selectivity of the FAMEWAX column separated all critical components.

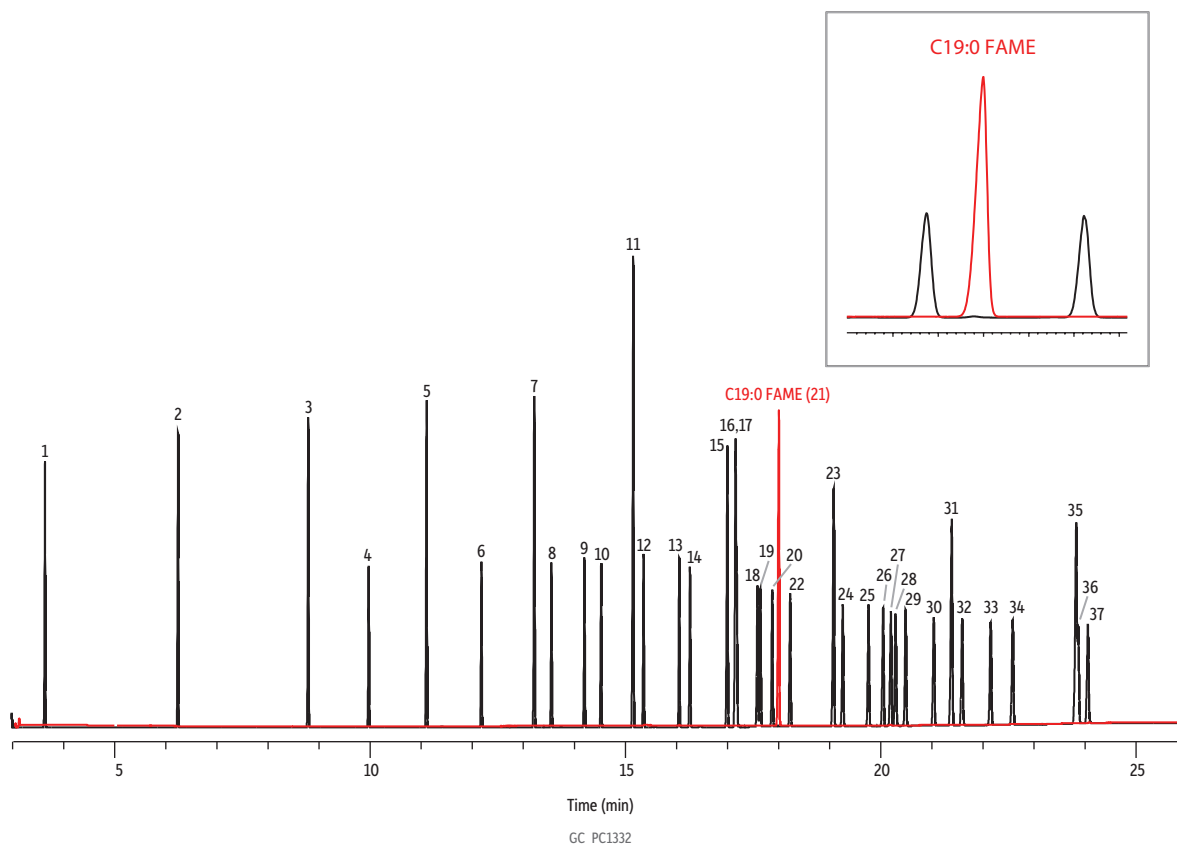
Figure 2: The Pro EZGC chromatogram modeler predicts good separation of the internal standard and linolenic acid methyl ester from FAMES that are commonly present in biodiesel using a FAMEWAX capillary column.



Peaks	t _r (min)	R _s	Peak Width (min)	T _{peak} (°C)	Peaks	t _r (min)	R _s	Peak Width (min)	T _{peak} (°C)
1. C6:0	3.35	96.8	0.027	73.5	18. C18:3 (all-cis-9,12,15)	17.90	5.1	0.041	209.5
2. C8:0	6.00	86.3	0.030	100.0	19. C20:0	18.74	3.9	0.043	213.7
3. C10:0	8.57	39.1	0.030	125.7	20. C20:1 (cis-11)	18.91	3.9	0.045	214.6
4. C11:0	9.75	37.3	0.030	137.5	21. C20:2 (all-cis-11,14)	19.40	6	0.046	217.0
5. C12:0	10.88	37.3	0.031	148.8	22. C20:3 (all-cis-8,11,14)	19.68	3.3	0.047	218.4
6. C14:0	12.98	10.5	0.031	169.8	23. C21:0	19.84	1.6	0.047	219.2
7. C14:1 (cis-9)	13.31	10.5	0.032	173.1	24. C20:4 (all-cis-5,8,11,14)	19.92	1.6	0.048	219.6
8. C16:0	14.91	6.2	0.032	189.1	25. C20:3 (all-cis-11,14,17)	20.11	4.1	0.048	220.6
9. C16:1 (cis-9)	15.11	6.2	0.032	191.1	26. C20:5 (all-cis-5,8,11,14,17)	20.65	7.2	0.050	223.2
10. C17:0	15.80	21.7	0.032	198.0	27. C22:0	21.01	3.8	0.050	225.1
11. C18:0	16.72	4.3	0.035	203.6	28. C22:1 (cis-13)	21.20	3.8	0.051	226.0
12. C18:1 (cis-9)	16.87	0.6	0.036	204.3	29. C22:2 (all-cis-13,16)	21.75	10.7	0.052	228.7
13. C18:1 (trans-9)	16.89	0.6	0.036	204.4	30. C24:0	23.40	0.4	0.054	237.0
14. C18:2 (all-cis-9,12)	17.28	1.4	0.038	206.4	31. C22:6 (all-cis-4,7,10,13,16,19)	23.43	0.4	0.056	237.1
15. C18:2 (all-trans-9,12)	17.34	1.4	0.038	206.7	32. C24:1 (cis-15)	23.62	3.5	0.055	238.1
16. C18:3 (all-cis-6,9,12)	17.56	3.4	0.039	207.8					
17. C19:0	17.70	3.4	0.039	208.5					

Column: FAMEWAX, 30.00 m, 0.25 mm ID, 0.25 µm (cat.# 12497)
 Carrier Gas: Hydrogen, Constant Flow @ 1.75 mL/min
 Average Velocity: 48.32 cm/sec
 Outlet Pressure (abs): 14.70 psi (Atmospheric Pressure)
 Oven Temp: 60 °C (hold 2 min) to 200 °C @ 10 °C/min to 240 °C @ 5 °C/min

Figure 3: Chromatogram overlay of a FAME standard and a C19:0 internal standard analyzed on a FAMEWAX column closely match modeled results.



Peaks	tr (min)	Conc. (mg/mL)	Structural Nomenclature
1. Methyl capronate	3.629	1.2	C6:0
2. Methyl caprylate	6.237	1.2	C8:0
3. Methyl caprate	8.787	1.2	C10:0
4. Methyl undecanoate	9.971	0.6	C11:0
5. Methyl laurate	11.105	1.2	C12:0
6. Methyl tridecanoate	12.179	0.6	C13:0
7. Methyl myristate	13.215	1.2	C14:0
8. Methyl myristoleate	13.549	0.6	C14:1 (cis-9)
9. Methyl pentadecanoate	14.196	0.6	C15:0
10. Methyl pentadecenoate	14.524	0.6	C15:1 (cis-10)
11. Methyl palmitate	15.152	1.8	C16:0
12. Methyl palmitoleate	15.355	0.6	C16:1 (cis-9)
13. Methyl margarate	16.052	0.6	C17:0
14. Methyl heptadecenoate	16.261	0.6	C17:1 (cis-10)
15. Methyl stearate	16.995	1.2	C18:0
16. Methyl oleate	17.156	1.2	C18:1 (cis-9)
17. Methyl elaidate	17.168	1.2	C18:1 (trans-9)
18. Methyl linoleate	17.583	0.6	C18:2 (all-cis-9,12)
19. Methyl linolelaidate	17.641	0.6	C18:2 (all-trans-9,12)
20. Methyl γ-linolenate	17.874	0.6	C18:3 (all-cis-6,9,12)
21. Methyl nonadecanoate	18.052	2.0	C19:0
22. Methyl α-linolenate	18.223	0.6	C18:3 (all-cis-9,12,15)
23. Methyl arachidate	19.075	1.2	C20:0
24. Methyl (Z)-11-eicosenoate	19.255	0.6	C20:1 (cis-11)
25. Methyl 11,14-eicosadienoate	19.761	0.6	C20:2 (all-cis-11,14)
26. Methyl eicosa-8,11,14-trienoate	20.046	0.6	C20:3 (all-cis-8,11,14)
27. Methyl heneicosanoate	20.197	0.6	C21:0
28. Methyl arachidonate	20.290	0.6	C20:4 (all-cis-5,8,11,14)
29. Methyl 11,14,17-eicosatrienoate	20.488	0.6	C20:3 (all-cis-11,14,17)
30. Methyl 5,8,11,14,17-eicosapentanoate	21.036	0.6	C20:5 (all-cis-5,8,11,14,17)
31. Methyl behenate	21.39	1.2	C22:0
32. Methyl erucate	21.595	0.6	C22:1 (cis-13)
33. Methyl docosadienoate	22.150	0.6	C22:2 (all-cis-13,16)
34. Methyl tricosanoate	22.584	0.6	C23:0
35. Methyl lignocerate	23.826	1.2	C24:0
36. Methyl docosahexaenoate	23.863	0.6	C22:6 (all-cis-4,7,10,13,16,19)
37. Methyl nervonate	24.055	0.6	C24:1 (cis-15)

Column FAMEWAX, 30 m, 0.25 mm ID, 0.25 μm (cat.# 12497)
Sample Food industry FAME mix (cat.# 35077)
Methyl nonadecanoate (cat.# 35055)
Standard cat.# 35055 was dissolved in toluene.

Diluent:
Injection 1 μL split (split ratio 100:1)
Liner: Topaz 4.0 mm ID Precision inlet liner w/wool (cat.# 23305)
Inj. Temp.: 240 °C
Oven
Oven Temp.: 60 °C (hold 2 min) to 200 °C at 10 °C/min to 240 °C at 5 °C/min (hold 7 min)

Carrier Gas Hz, constant flow
Flow Rate: 1.7 mL/min
Detector FID @ 250 °C
Instrument Agilent 7890B GC

Notes
This chromatogram is an overlay of two injections: food industry FAME standard (black) and C19:0 methyl ester in toluene (red). An excellent separation of C19:0 (used in EN 14:103 as an internal standard) and the most prevalent FAMES found in biodiesel blends was achieved. Note that C4:0 from the food industry FAME standard elutes in the solvent front.

After empirically demonstrating that good separation of FAMES in the reference standard was achieved using a FAMEWAX column, commercially obtained biodiesel samples were also analyzed according to method EN14103. Good chromatographic results were obtained as shown in Figures 4 and 5. In addition, repeatability was assessed in order to evaluate the potential for carryover or poor sample transfer onto the column. Calculations of total ester content and the linolenic acid methyl ester content (all isomers combined) were highly repeatable, indicating consistent chromatographic performance and no observable issues with carryover or sample transfer for the analysis of FAMES in biodiesel (Table I).

Figure 4: Chromatographic analysis of canola biodiesel according to method EN 14103 (2011). The red overlay is a linolenic acid methyl ester isomer mix standard (Sigma-Aldrich, L6031-25 mg) showing that all the isomers are separated from the C19:0 internal standard.

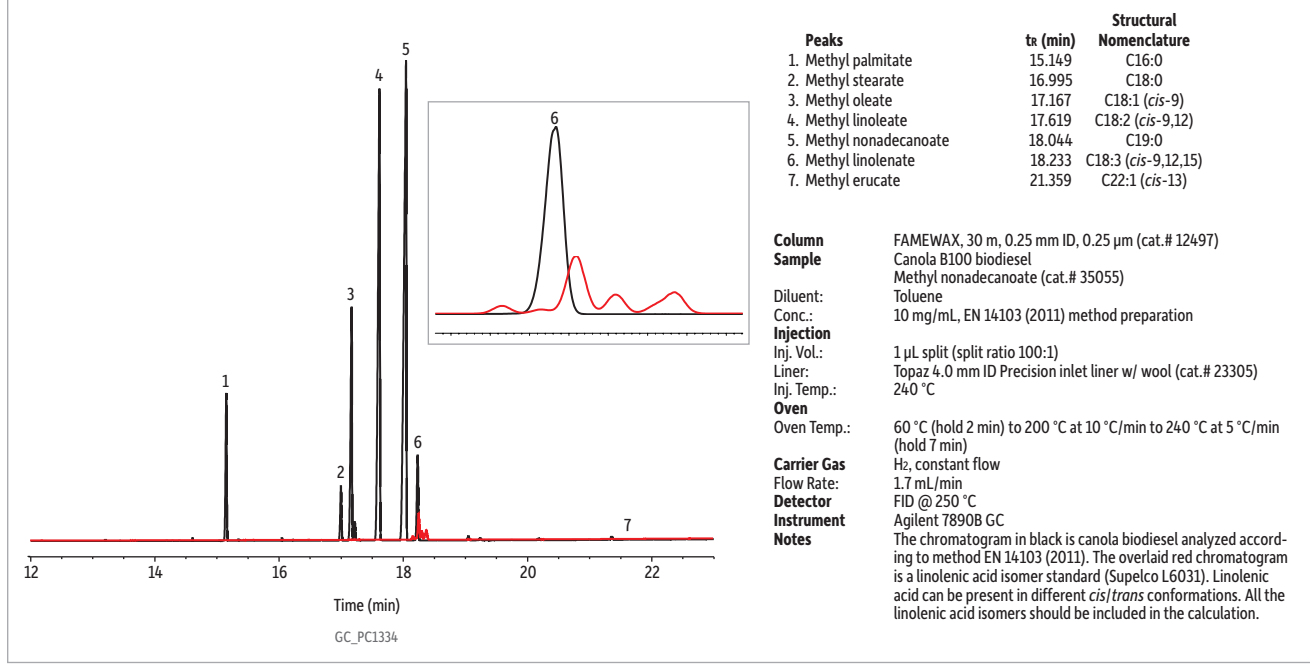


Figure 5: Chromatographic analysis of soy biodiesel according to method EN 14103 (2011).

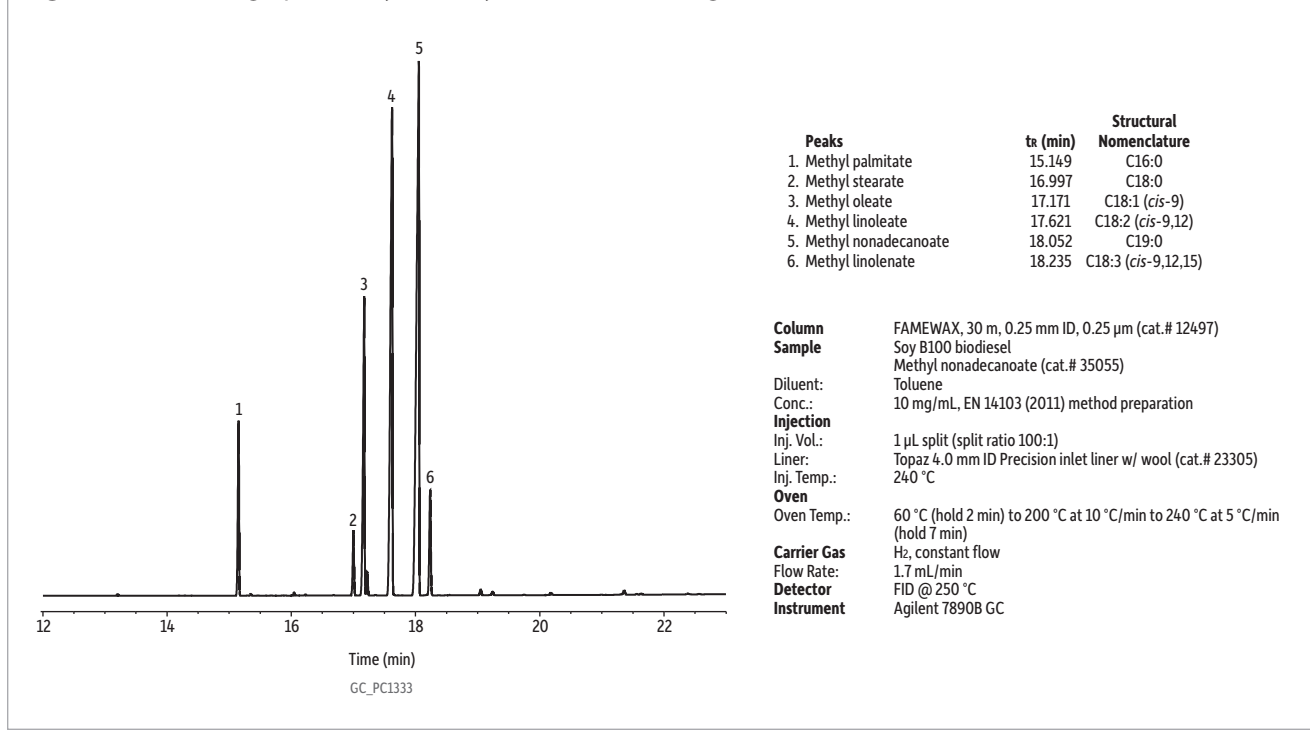


Table I: Total FAME and linolenic methyl ester weight percents with analysis of precision.

	Soy B100		Canola B100	
	Total FAME (wt%)	C18:3 (wt%)	Total FAME (wt%)	C18:3 (wt%)
Run 1	90.69	7.32	92.70	7.39
Run 2	90.70	7.32	92.76	7.39
Run 3	90.64	7.32	92.68	7.39
Run 4	90.65	7.32	92.74	7.39
Run 5	90.66	7.32	92.78	7.40
Run 6	90.55	7.31	92.61	7.39
Run 7	90.64	7.31	92.62	7.39
%RSD	0.05	0.05	0.07	0.05

Conclusion

Using the Pro EZGC chromatogram modeler was a quick and easy way of identifying a column that would successfully analyze FAMES in biodiesel, without spending any money or any time in the lab. While this work used the analytical conditions described in method EN 14103, the Pro EZGC modeler also can be used to further optimize these conditions and achieve a faster analysis while still maintaining resolution between all the targeted compounds. Empirical analysis of FAMES in biodiesel samples confirmed that the selectivity of the FAMEWAX column allowed all critical compounds to be separated. Excellent peak shape and low bleed provided accurate, precise, and repeatable quantification of analytes.

References

- (1) F. Gunstone, The chemistry of oils and fats: sources, composition, properties and uses, Wiley-Blackwell, 2009. <https://www.wiley.com/The+Chemistry+of+Oils+and+Fats%3A+Sources%2C+Composition%2C+Properties+and+Uses-p-9781405150026>
- (2) DIN EN 14103, Fat and oil derivatives - Fatty Acid Methyl Esters (FAME) - Determination of ester and linolenic acid methyl ester contents, 2011. <https://www.din.de/en/getting-involved/standards-committees/nmp/wdc-beuth:din21:232191873>
- (3) J. Pullen, K. Saeed, An overview of biodiesel oxidation stability, Renewable and Sustainable Energy Reviews 16 (2012) 5924-5950. <https://doi.org/10.1016/j.rser.2012.06.024>

Food Industry FAME Mix (37 components)

Chain, Compound (CAS#), % by Weight

C4:0 Methyl butyrate (623-42-7), 4%
 C6:0 Methyl caproate (106-70-7), 4%
 C8:0 Methyl caprylate (111-11-5), 4%
 C10:0 Methyl decanoate (110-42-9), 4%
 C11:0 Methyl undecanoate (1731-86-8), 2%
 C12:0 Methyl dodecanoate (111-82-0), 4%
 C13:0 Methyl tridecanoate (1731-88-0), 2%
 C14:0 Methyl myristate (124-10-7), 4%
 C14:1 (*cis*-9) Methyl myristoleate (56219-06-8), 2%
 C15:0 Methyl pentadecanoate (7132-64-1), 2%
 C15:1 (*cis*-10) Methyl pentadecenoate (90176-52-6), 2%
 C16:0 Methyl palmitate (112-39-0), 6%
 C16:1 (*cis*-9) Methyl palmitoleate (1120-25-8), 2%
 C17:0 Methyl heptadecanoate (1731-92-6), 2%
 C17:1 (*cis*-10) Methyl heptadecenoate (75190-82-8), 2%
 C18:0 Methyl stearate (112-61-8), 4%
 C18:1 (*trans*-9) Methyl octadecenoate (1937-62-8), 2%
 C18:1 (*cis*-9) Methyl oleate (112-62-9), 4%
 C18:2 (all-*trans*-9,12) Methyl linolelaidate (2566-97-4), 2%
 C18:2 (all-*cis*-9,12) Methyl linoleate (112-63-0), 2%
 C18:3 (all-*cis*-6,9,12) Methyl linolenate (16326-32-2), 2%
 C18:3 (all-*cis*-9,12,15) Methyl linolenate (301-00-8), 2%
 C20:0 Methyl arachidate (1120-28-1), 4%
 C20:1 (*cis*-11) Methyl eicosenoate (2390-09-2), 2%
 C20:2 (all-*cis*-11,14) Methyl eicosadienoate (2463-02-7), 2%
 C20:3 (all-*cis*-8,11,14) Methyl eicosatrienoate (21061-10-9), 2%
 C20:3 (all-*cis*-11,14,17) Methyl eicosatrienoate (55682-88-7), 2%
 C20:4 (all-*cis*-5,8,11,14) Methyl arachidonate (2566-89-4), 2%
 C20:5 (all-*cis*-5,8,11,14,17) Methyl eicosapentaenoate (2734-47-6), 2%
 C21:0 Methyl heneicosanoate (6064-90-0), 2%
 C22:0 Methyl behenate (929-77-1), 4%
 C22:1 (*cis*-13) Methyl erucate (1120-34-9), 2%
 C22:2 (all-*cis*-13,16) Methyl docosadienoate (61012-47-3), 2%
 C22:6 (all-*cis*-4,7,10,13,16,19) Methyl docosahexaenoate (2566-90-7), 2%
 C23:0 Methyl tricosanoate (2433-97-8), 2%
 C24:0 Methyl lignocerate (2442-49-1), 4%
 C24:1 (*cis*-15) Methyl nervonate (2733-88-2), 2%

30 mg/mL total in methylene chloride, 1 mL/ampul cat.# 35077 (ea.)

Quantity discounts not available.

No data pack available.

Neat Fatty Acid Methyl Esters

Use these materials to prepare specific mixtures not commercially available. These products are of the highest purity available, typically 99% by GC-FID analysis. Each compound is packaged under a nitrogen blanket to ensure product stability. A certificate of analysis is provided with each ampul.



FAMEWAX Columns (USP G16) (fused silica)

polar phase; Crossbond polyethylene glycol

Description	temp. limits	qty.	cat.#
FAMEWAX 30 m, 0.25 mm ID, 0.25 µm	20 to 240/250 °C	ea.	12497
FAMEWAX 30 m, 0.32 mm ID, 0.25 µm	20 to 240/250 °C	ea.	12498
FAMEWAX 30 m, 0.53 mm ID, 0.50 µm	20 to 250 °C	ea.	12499



Chain	Description	CAS #	qty.	cat.#
C6:0	Methyl caproate	106-70-7	ea.	35037
C8:0	Methyl caprylate	111-11-5	ea.	35039
C10:0	Methyl caprate	110-42-9	ea.	35041
C11:0	Methyl undecanoate	1731-86-8	ea.	35042
C12:0	Methyl laurate	111-82-0	ea.	35043
C13:0	Methyl tridecanoate	1731-88-0	ea.	35044
C14:0	Methyl myristate	124-10-7	ea.	35045
C16:0	Methyl palmitate	112-39-0	ea.	35048
C16:1 Δ 9 <i>cis</i>	Methyl palmitoleate	1120-25-8	ea.	35049
C17:0	Methyl heptadecanoate	1731-92-6	ea.	35050
C18:0	Methyl stearate	112-61-8	ea.	35051
C18:1 Δ 9 <i>cis</i>	Methyl oleate	112-62-9	ea.	35052
C18:2 Δ 9,12 <i>cis</i>	Methyl linoleate	112-63-0	ea.	35053
C18:3 Δ 9,12,15 <i>cis</i>	Methyl linolenate	301-00-8	ea.	35054
C19:0	Methyl nonadecanoate	1731-94-8	ea.	35055
C20:0	Methyl arachidate	1120-28-1	ea.	35056
C20:1 Δ 11 <i>cis</i>	Methyl eicosenoate	2390-09-2	ea.	35057
C20:3 Δ 11,14,17 <i>cis</i>	Methyl eicosatrienoate	55682-88-7	ea.	35059
C21:0	Methyl heneicosanoate	6064-90-0	ea.	35061
C22:0	Methyl behenate	929-77-1	ea.	35062
C22:1 Δ 13 <i>cis</i>	Methyl erucate	1120-34-9	ea.	35063
C24:0	Methyl lignocerate	2442-49-1	ea.	35064

Quantity discounts not available.

No data pack available.



Questions? Contact us or your local Restek representative (www.restek.com/contact-us).

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