Analysis of Fatty Acid Methyl Ester (FAME) Content and Distribution in Biodiesel Blends Using Heart-Cutting 2D Gas Chromatography

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The analysis of the fatty acid methyl ester (FAME) content in blended biodiesel samples is described using a heartcutting two-dimensional (2D) gas chromatographic (GC) system. A Capillary Flow Technology Deans switch is used to interface a primary nonpolar capillary column to a secondary polar capillary column. The primary column separates most of the petroleum hydrocarbons from the FAMEs. The FAMEs are selectively transferred to the secondary column, where they are completely resolved from the remaining hydrocarbon matrix. The instrument is calibrated using the total response of all separated FAME peaks over a range of 1 to 25 volume percent. After calibration, a sample of commercially blended B20 biodiesel is analyzed; the results show excellent quantitative precision. The distribution of individual FAMEs is also determined and the results show that the commercial sample contains biodiesel made from soybean oil. The separation of palm oil and coconut oil FAMEs in biodiesel blends is also demonstrated using the heart-cutting 2D GC approach.

Introduction

High crude oil prices combined with disruptions in supply and refining capacity have driven the price of motor fuels to new highs and created spot shortages throughout the world. This has given new urgency to the development of locally produced alternative renewable fuels. This effort offers the potential to reduce reliance on crude oil as well as lower emissions of airborne pollutants and greenhouse gases.

Biodiesel is a motor or heating fuel produced from renewable vegetable oils derived from crops such as sunflower, soybean, rapeseed, and palm. Biodiesel is made by transesterification of vegetable oil or animal fats to produce a mixture of fatty acid methyl esters (FAMEs). Pure biodiesel is called B100 and must meet industry standard specifications before it can be used as a fuel or blending stock. The distribution of FAMEs in a B100 mixture depends on the feedstock source as shown in *Table 1*.[1] The relative amounts of FAMEs in biodiesel can vary widely and have different effects on both the fuel and handling properties.[2]

Pure biodiesel is a relatively simple mixture and gas chromatography (GC) is routinely used to test product quality. Commercially, pure biodiesel is blended with no. 2 petroleum diesel to create a motor fuel with 1 to 20 volume percent (vol%) of total FAME content. These blends are designated B1 to B20, respectively. As a blend, it is difficult to quantify the FAME content in the presence of the petroleum hydrocarbons using conventional capillary GC. EN14331 is the only industry standard GC method for measuring the FAME content in biodiesel blends.[3] This method requires atmospheric pressure silica-column liquid chromatography (LC) to physically separate the petroleum diesel from the FAMEs in the sample. The FAME fractions from the silica column are then analyzed using GC. This method is time-consuming and is only scoped for 5 vol% (B5) or lower biodiesel blends.

Two-dimensional (2D) GC offers a higher resolution solution to the analysis of very complex mixtures. The most widely practiced 2D GC technique is called heart-cutting. Selected, unresolved peaks are transferred from one column to another column of different selectivity where a second separation takes place. By carefully choosing the columns and instrument conditions, it is possible to obtain higher resolution for several compounds in a complex mixture. A device commonly used to transfer peaks from one column to the next is a Deans switch. Due to improvements in GC hardware, there has been renewed interest in heart-cutting methods for the analysis of petroleum and petrochemical products.[4–7] Recently a new type of Deans switch has been developed using Capillary Flow Technology to further improve the precision and performance of heart-cutting 2D GC.[7, 8] This application describes a new method using a Capillary Flow Technology Deans switch to separate the FAME compounds in biodiesel blends.

Experimental

An Agilent 7890A GC was equipped according to the details outlined in *Table 2*. After column installation, the GC conditions were set according to the data in *Table 3*. Instrument pressures, flow rates, and the fixed restrictor dimensions were determined using a Deans switch calculator software program designed for this system. This calculator program is included with the Deans switch hardware option for the Agilent 7890A GC.

Determining Cut Times and System Calibration

A low erucic rapeseed oil reference standard was used to determine retention times and cut times on the HP-5ms column. This standard was dissolved in 5 mL of hexane containing 10 mg/mL of methyl heneicosanoate (C21:0) as the internal standard. The standard was injected with the Deans switch set in the off position during the entire run. This same standard was then run using these cut times to determine the retention time of each FAME peak on the HP-INNOWax column. Alternatively, a sample of the biodiesel blending stock could be used as a standard for determining heart-cut times.

Once the retention times and cut times for each FAME group were determined, a matrix blank was run using these cut times. This will determine if there is any potential interference from the matrix that is not resolved by the secondary column. For this work, a no. 2 diesel fuel containing no biodiesel was used as the matrix. System Calibration and Sample Analysis Calibration standards were prepared by mixing no. 2 diesel fuel with a commercially available B100 soybean biodiesel in 12-mL vials equipped with Teflon-lined caps. Standards were made to represent 1, 2, 5, 10, and 25 vol% biodiesel blends. A commercially blended soybean B20 fuel was obtained from Uncle Willie's Deli

Fatty Acid Distribution												
Oil Type	C8:0	C10:0	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0 C22:0	C20:1 C22:1
Soybean				0.3	7–11	0–1	3–6	22–34	50–60	2–10	5–10	
Rapeseed					2–5	0.2	1–2	10–15	10–20	5–10	0.9	50–60
Palm				1–6	32–47		1–6	40–52	2–11			
Coconut	5–9	4–10	44–51	13–18	7–10		1–4	5–8	1–3			

Table 1. Fatty Acid Distribution of Common Biodiesel Feedstocks

Standard 7890A GC hardware	
G3440A	Agilent 7890A Series GC
Option 112	Capillary split/splitless inlet with EPC control
Option 211	(2 of each) Capillary FID with EPC control
Option 309	Pneumatics control module with EPC control
Option 888	Factory installed Capillary Flow Technology Deans Switch
G2613A	Agilent 7683 autoinjector
Columns	
Primary column	HP-5ms, 15 m × 0.25 mm id × 0.1 µm (part no. 19091S-331)
Secondary column	HP-INNOWax, 30 m × 0.25 mm id × 0.5 µm (part no. 19091N-233)
Deans restrictor	Deactivated fused silica tubing, 0.77 m × 0.1 mm id (part no. 160-2635-5)
Data system	
G2070	Agilent multi-technique ChemStation
Optional consumables	
5181-1267	10 µL Teflon fixed autoinjector syringe
5183-4647	Inlet liner optimized for split operation
Standards	
H3265-100MG*	Methyl heneicosanoate (C21:0)
O7756-1AMP*	Low erucic rapeseed oil reference standard, 100 mg

*Available from Sigma-Aldrich, PO Box 14508, St. Louis, MO 63178, USA

Table 2. System Configuration

Injection port	Split mode, 200:1 split ratio
Temperature	250 °C
EPC pressure	33.86 psi helium, constant pressure mode
Injection size	0.2 µL
HP-5ms column flow	1.5 mL/min
Pneumatics control module	30.70 psi helium, constant pressure mode
HP-INNOWax column flow	3.5 mL/min
FID temperatures	275 °C
Oven temperature program	
Initial temperature	50 °C for 0 min
Ramp number 1	20 °C/min to 210 °C for 18 min
Ramp number 2	20 °C/min to 230 °C for 13 min

Table 3. Instrument Conditions

& Fuel (Woodside, DE, USA) for use as a test sample. A 10 mg/mL solution of methyl heneicosanoate (C21:0) in chromatographic-grade hexane was prepared for use as an internal standard. Each calibration standard and sample was prepared for GC analysis by weighing a 250-mg aliquot and adding 1 mL of the internal standard solution. After calibration, the B20 biodiesel sample was analyzed as a performance check of the system.

Results and Discussion

The HP-5ms primary column separation of the FAMEs in the rapeseed oil reference sample is shown in *Figure 1*. The HP-5ms column does not completely separate the individual FAMEs; however, they generally are separated by groups according to the number of carbons in the fatty acid chain. Figure 1 also shows a chromatogram of pure no. 2 diesel fuel on the HP-5ms column. Most of the hydrocarbons elute before the first FAME peak, methyl myristate (C14:0). Therefore, co-elution of FAMEs and hydrocarbons primarily occurs between 9 and 15 minutes on the HP-5ms column.

The heart-cut times of each FAME group were determined from the data shown in *Figure 1*. The HP-5ms retention times, the heart-cut times, and the HP-INNOWax retention times are summarized in *Table 4*. Due to slight variations in columns and hardware, the retention times and cut times listed in *Table 4* cannot be used for every system. Instead, each analyst must determine the correct heart-cut times and secondary column retention times for their system.

After heart-cutting, the FAME peaks are transferred from the HP-5ms column to the secondary HP-INNOWax column, where most are further resolved into their individual components as shown in *Figure 2*. However, for the C20 group and C22 group, resolution was lost within each group after heart-cutting to the HP-INNOWax column. For analysts who prefer to maintain the separation within these two groups, it is not necessary to use heart-cutting since these FAMEs elute after the petroleum hydrocarbons on the primary HP-5ms column.

Using the heart-cut times obtained from the previous experiment, a sample of pure no. 2 diesel was run to observe any potential matrix interference with the FAMEs on the secondary column. *Figure 3* shows a comparison of the FAME separation and the matrix hydrocarbons on the HP-INNOWax column. For the major FAME components found in biodiesel, there are no significant co-elutions with petroleum hydrocarbons on the INNOWax column after heart-cutting. Due to variations in composition of different types of petroleum diesel fuel, practitioners of this method should perform this experiment using the no. 2 diesel fuel found in their blends.

The system was calibrated for quantitative analysis by running the standards described in the experimental section. Since the total amount of biodiesel in the blends is distributed among several FAME peaks, it is not possible to use any single peak for quantification. Instead, the area responses of all FAME peaks are summed to represent the total amount of biodiesel in the blend for calibration. This operation is accomplished using the peak grouping calibration functions in the Agilent ChemStation.

A least-squares linear calibration curve was prepared using the summed area response of all FAME peaks relative to the area response of the C21:0 internal standard. The calibration curve shows good linearity for biodiesel blends containing total FAME concentrations from 1 vol% to 25 vol% (*Figure 4*). The commercially blended biodiesel sample was run five times after calibration and one of the chromatograms is shown in *Figure 5*. The results summarized in *Table 5* show the commercial sample contained a total FAME content of 20.5%, which confirms the sample as a B20 biodiesel blend.

Since this method can identify individual FAMEs in a biodiesel blend, it is possible to determine the relative distribution of the esters in the fuel. This data can be useful in determining the type of feedstock used to make the B100 blending stock. The identity and distribution of FAMEs found in the B20 biodiesel blend sample indicates that soybean oil was the biodiesel feedstock (*Table 6*).

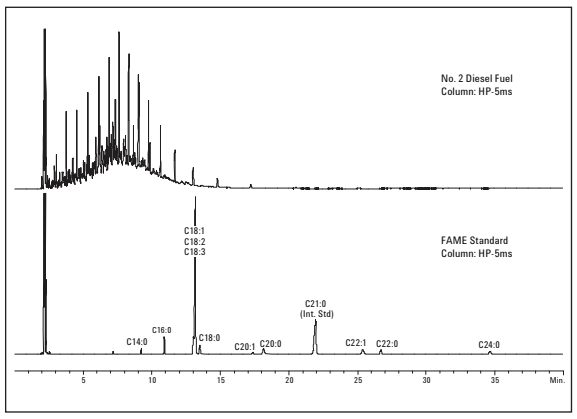

Figure 1. The lower chromatogram shows the separation of FAMEs on the primary HP-5ms column. The upper chromatogram shows the separation of pure no. 2 diesel fuel on the same column. A blended biodiesel fuel containing FAMEs and no. 2 diesel fuel would have unresolved compounds between 2 and 15 minutes on this column.

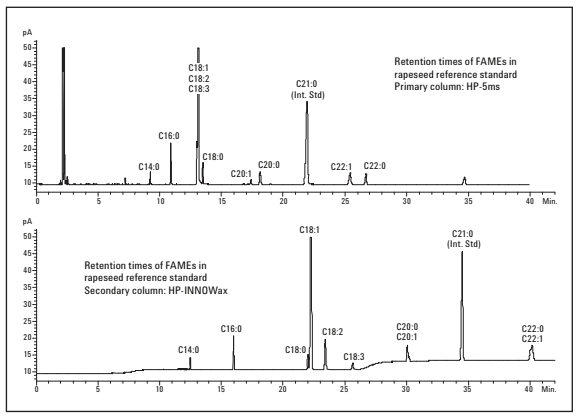

Figure 2. The upper chromatogram shows the separation of FAMEs on the primary HP-5ms column before heart-cutting. After heart-cutting, the lower chromatogram shows the separation of the FAMEs on the HP-INNOWax secondary column.

FAME	Carbon number	HP-5ms RT (min.)	Cut time (min)	HP-INNOWax RT (min)
Methyl-myristate	C14:0	9.21	9.10 – 9.28	12.46
Methyl-palmitate	C16:0	10.90	10.77 – 11.02	15.99
Methyl linolenate	C18:3	13.03	12.85 – 13.60	25.63
Methyl-oleate	C18.1	13.16	12.85 – 13.60	22.26
Methyl-linoelate	C18.2	13.16	12.85 – 13.60	23.41
Methyl stearate	C18:0	13.49	12.85 – 13.60	22.00
Methyl-eicosanoate	C20:1	17.35	17.14 – 17.50	30.18
Methyl-arachidate	C20:0	18.13	17.90 – 18.31	30.10
Methyl-heneicosanoate (Int. Std.)	C21:0	21.94	21.53 – 22.25	34.49
Methyl-erucate	C22:1	25.40	25.06 – 26.43	40.18
Methyl-behenate	C22:0	26.70	26.43 – 26.91	40.18

Table 4. Cut Times for C14 to C22 FAMES as Shown in Figures 1 and 2

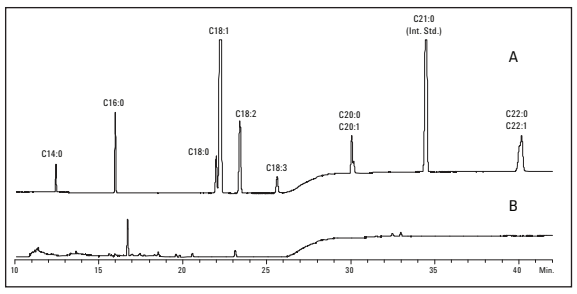

Figure 3. The upper chromatogram (A) shows the retention times of FAMEs on the secondary HP-INNOWax column after heart-cutting. The lower chromatogram (B) shows the hydrocarbon matrix of no. 2 diesel fuels after heart-cutting. No large peaks from the hydrocarbon matrix were found to co-elute with the FAME peaks after heart-cutting to the HP-INNOWax column.

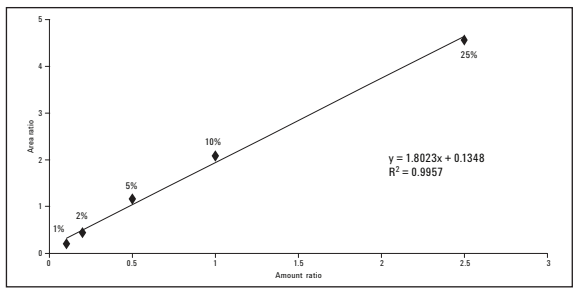

Figure 4. A calibration curve for biodiesel blends containing soybean biodiesel between 1 vol% (B1) and 25 vol% (B25). This calibration was prepared using the total peak areas of all FAMEs found in the blends.

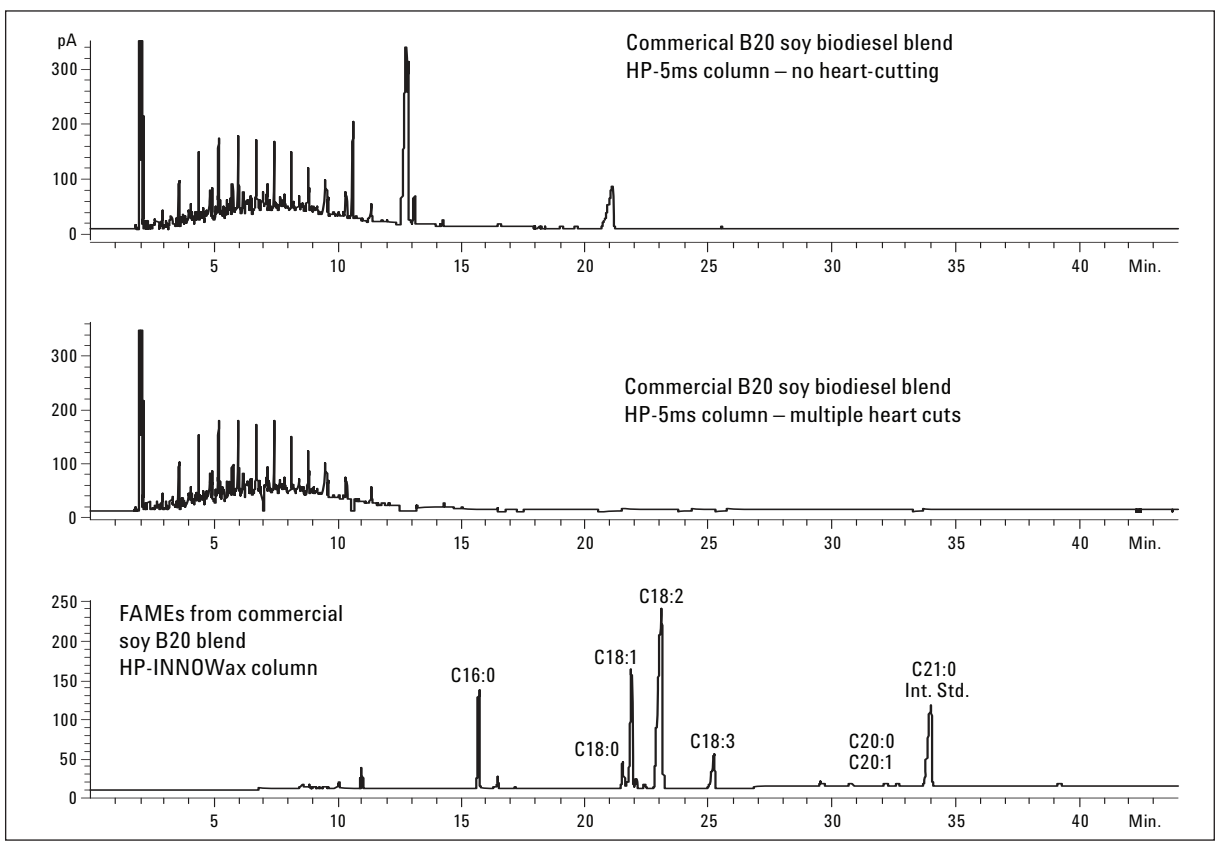

Figure 5. A commercially prepared B20 sample containing soybean biodiesel was analyzed using heart-cutting 2D GC. The upper chromatogram shows the primary column separation before heart-cutting. The middle and lower chromatograms show the sample after heart-cutting the FAMEs from the HP5ms column to the HP-INNOWax column.

Run	Volume %
1	20.4
2	20.5
3	20.5
4	20.5
5	20.5
Average	20.5
RSD (%)	0.1

Table 5. Analysis of a B20 (20 vol%) Soybean Biodiesel Commercial Blend

This heart-cutting 2D GC technique can also be used for measuring FAMEs in other types of biodiesel blends. In many regions throughout the world, tropical vegetable oils such as palm and coconut are used to make B100 biodiesel. Biodiesel blends made from these tropical oils can also be analyzed using this method. This is demonstrated in *Figures 6 and 7*, where B20 blends containing palm biodiesel and coconut biodiesel are measured using this method. The FAMEs derived from palm oil are somewhat less complicated than those derived from soybean. Methyl palmitate is the major peak. No methyl linolenate (C18:3) or C20 FAMEs were found in the sample.

Coconut oil contains a wider and lighter range of fatty acids as shown in *Table 1*. The complexity of coconut biodiesel results in more co-elution of FAMEs with hydrocarbons in blended fuels. However, the heart-cutting 2D GC technique used in this method can successfully separate coconut methyl ester from these hydrocarbons, as shown in *Figure 7*.

Conclusions

The analysis of biodiesel content in biodiesel blends is a unique challenge due to the complexity of the sample. Conventional single capillary column GC cannot provide sufficient resolution to quantify the biodiesel using a universal detector such as an FID. Multidimensional GC provides a powerful tool for measuring both the total amount of biodiesel in the blend as well as the distribution of biodiesel FAMEs. A Capillary Flow Technology Deans switch can precisely heart-cut biodiesel FAMEs from a nonpolar column to a polar column. The first-dimension column separates the bulk of the hydrocarbons from the FAMEs. The secondary column resolves the FAMEs from co-eluting hydrocarbons and further separates individual FAME peaks. The improved chromatographic resolution of this technique allows the quantitative analysis of the total biodiesel content in a blend as well as the distribution of FAMEs contained in the sample.

References

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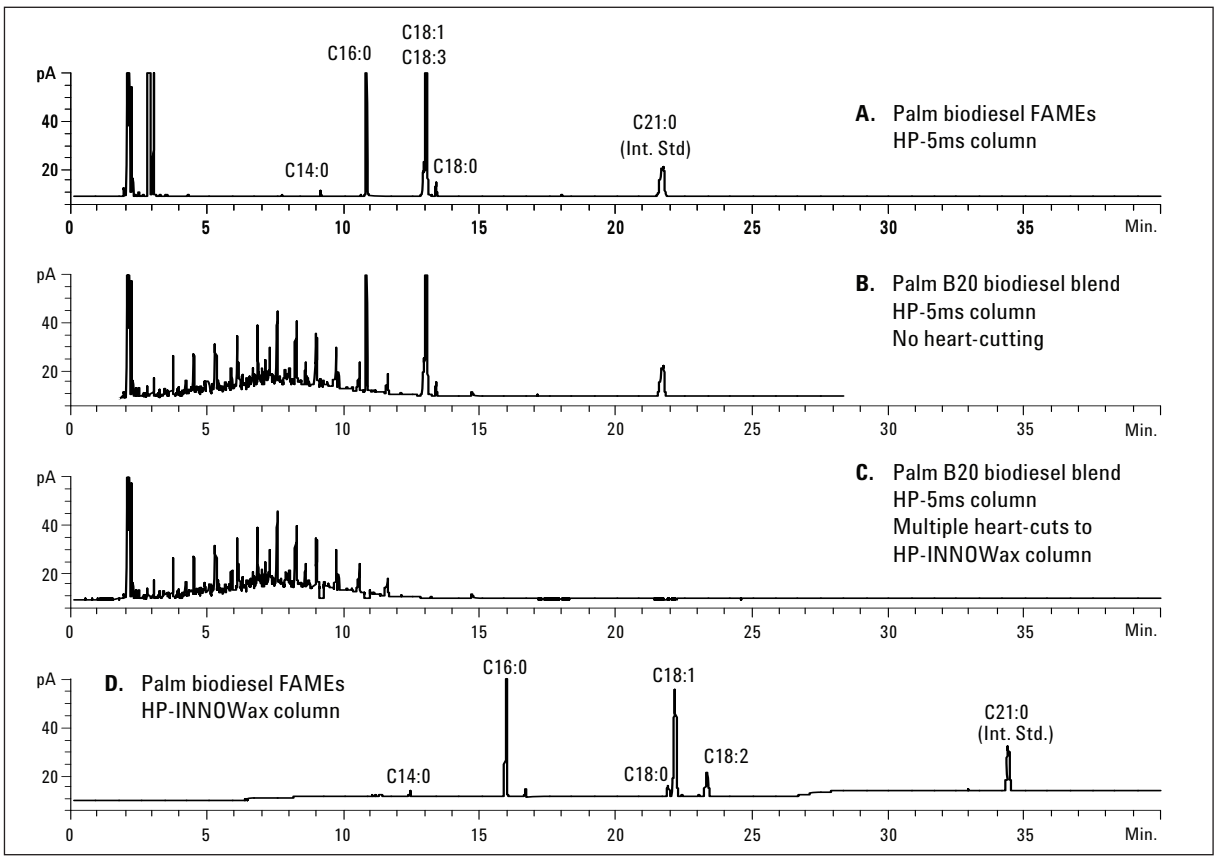

Figure 6. (A) Palm oil FAMEs on the primary HP-5ms column. (B) Palm B20 biodiesel blend with no heart-cutting. (C and D) Complete separation of palm FAMEs in B20 biodiesel blend using heart-cutting 2D GC.

Mass Fraction of FAME in Biodiesel						
	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0, C20:1
% found inB20 sample	11	4	22	53	8	2
% expected in soy	7–11	3–6	22–34	50–60	2–10	5–10

Table 6. Distribution of FAMEs Found in B20 Bodiesel Blend

FAME	Carbon number	HP-5ms RT (min)	Cut-time (min)	HP-INNOWax RT (min)
Methyl-caprylate	C8:0	4.72	4.68 – 4.77	6.62
Methyl-decanoate	C10:0	6.30	6.26 – 6.35	8.27
Methyl-laurate	C12:0	7.77	7.71 – 7.85	10.05
Methyl-myristate	C14:0	9.18	9.11 – 9.25	12.44
Methyl-palmitate	C16:0	10.85	10.78 – 10.92	15.96
Methyl-linoleate	C18:2	13.03	12.85 – 13.16	23.36
Methyl-oleate	C18:1	13.03	12.85 – 13.16	22.15
Methyl stearate	C18:0	13.74	13.34 – 13.49	21.95
Methyl-heneicosanoate (Int. Std.)	C21:0	21.73	21.47 – 21.91	34.43

Table 7. Cut Times for C8 to C18 Coconut Oil FAMES as Shown in Figures 7

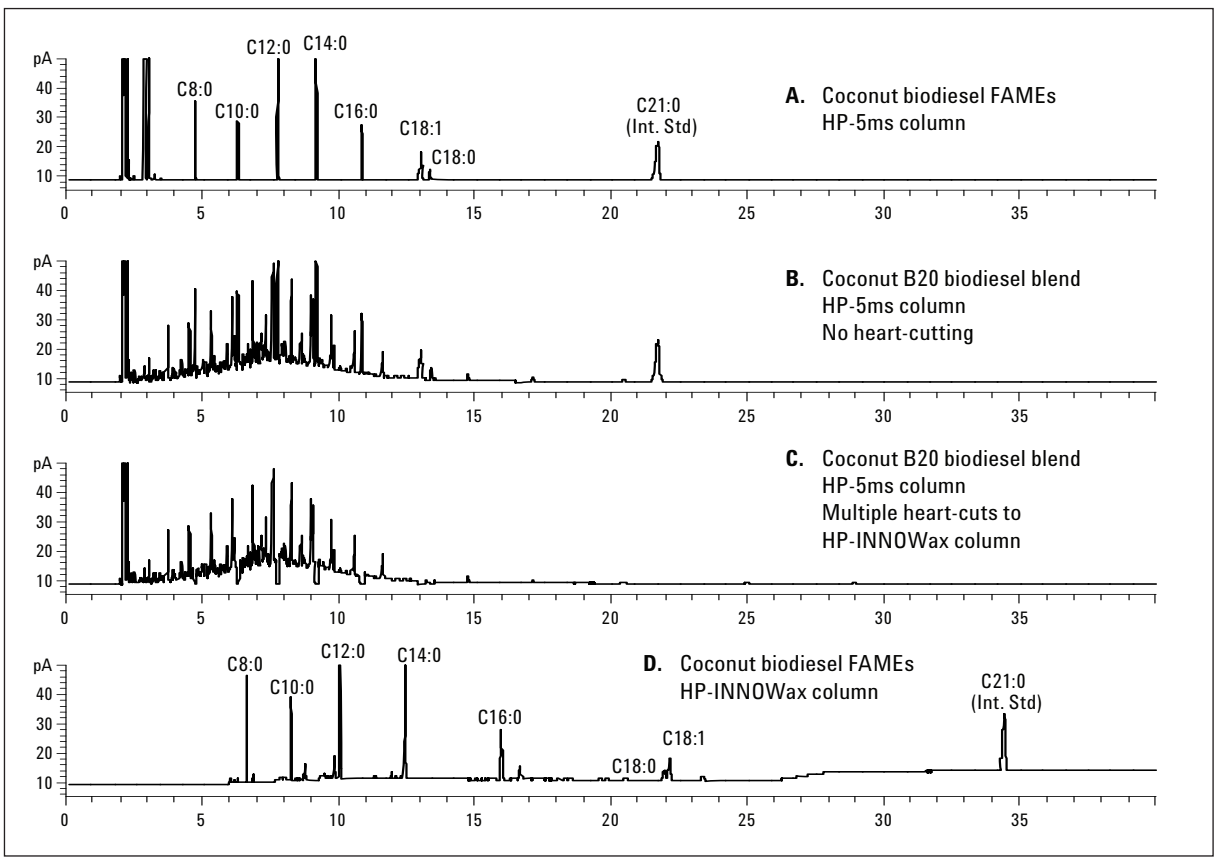

Figure 7. (A) Coconut oil FAMEs on the primary HP-5ms column. (B) Coconut B20 biodiesel blend with no heart-cutting. (C and D) Complete separation of coconut FAMEs in B20 biodiesel blend using heart-cutting 2D GC.