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Applikationsbericht

ACQUITY UPLC/ELS/UV: One Methodology for FFA, FAME, and TAG Analysis of Biodiesel

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Abstract

This application note focuses on the fatty acid methyl ester biodiesel production and describes a rapid analysis method using the Waters ACQUITY UPLC System with the Photodiode Array (PDA) and Evaporative Light Scattering (ELS) detectors with lower toxicity solvents, acetonitrile and 2-propanol, as the mobile phase.

Introduction

Biodiesel, either alone or with fossil diesel fuel, is gaining significant interest because of environmental factors, the upsurge in petroleum prices, financial incentives and government mandates for its use.[1–4] From 2004 to 2005, biodiesel production capacity in the United States increased from 0.1 million to 1.1 million tons. In 2004, the production capacity in the European Union was 2.3 million tons and is expected to increase to 4 million tons in 2007.[3] Biodiesel product standards have been established in various countries, including the U.S. (ASTM D 6751), EU (EN14214 and EN590), and Brazil (ANP 255).[1–4]

Defined as fatty acid methyl esters (FAME) of seed oils and animal fat, biodiesel is commonly produced by transesterification of triacylglycerols (TAG) with methanol in the presence of a catalyst (Figure 1). Potential contaminants of biodiesel products include unreacted TAG, reaction intermediates [mono-acylglycerols (MAG) and diacylglycerols (DAG)], reaction by-products (glycerol), and free fatty acids (FFA) from unwanted hydrolysis reactions. Contaminated biodiesel can lead to severe problems in trucks, automobiles and airplanes such as engine deposits, filter clogging, and fuel deterioration.1-2 To avoid this the production status is monitored to recognize and correct any problems at an early stage and also to quantify the contaminants in the final biodiesel product.[1,2,5,6]

Both GC and HPLC are used to analyze biodiesel and its contaminants[1,2,8-13] typically requiring multiple injections with different experimental conditions to characterize biodiesel and impurities. For example, FAME and TAG can be analyzed by GC. The TAG analysis requires a GC method with a special high temperature (350 °C) stable capillary column and derivatization of the non-volatile contaminants (FFA, MAG, and DAG) before injection. Derivatization is time-consuming and not always quantitative.[1,2,8] Most conventional HPLC methods have a 30 to 80 minute run time and use halogenated solvents that are known carcinogens, restricted, and sometimes prohibited in laboratories.[9–13]

This application note focuses on the fatty acid methyl ester biodiesel production and describes a rapid analysis method using the Waters ACQUITY UPLC System with the Photodiode Array (PDA) and Evaporative Light Scattering (ELS) detectors with lower toxicity solvents, acetonitrile and 2-propanol, as the mobile phase.

This 12-minute UPLC method enables high resolution and sensitive separation of biodiesel feedstock, reaction intermediates, glycerol, FFA, and the final products (FAME) in a single experiment. The ability to quickly and reliably analyze these critical components can facilitate monitoring the production processes to improve the yield. With better control of final product quality, the goals of successful commercialization and market acceptance are easier to reach.

Experimental

Sample Preparation

Biodiesel was synthesized using the kitchen biodiesel method with a supermarket brand soybean oil, reagent

grade MeOH and NaOH.[14] A small portion of biodiesel was diluted with IPA and the solution was filtered with a 0.45 µm PVDF syringe filter (WAT200531) to make a 12 mg/mL solution for UPLC analysis. Biodiesel related chemical standards (Table 1) were purchased from Sigma-Aldrich and TCI America and dissolved in 2-propanol (IPA) to make stock solutions. The stock solutions were further mixed to make a standard solution containing 0.5 mg/mL of standards and 0.7 mg/mL of soybean oil.

LC Conditions

LC system:	Waters ACQUITY UPLC with ACQUITY UPLC PDA/ELSD
Software:	Empower 2 (build 2154)
Detection:	PDA 195 to 300 nm
Sampling rate:	20 pts/s
Filter response:	fast
Weak wash:	2-propanol (600 µL)
Strong wash:	2-propanol (600 µL)
Seal wash:	90:10 water/CH_3CN (5 min)
Column temp.:	30 °C
Injection:	2 µL (full loop)
Mobile phase A:	CH_3CN
Mobile phase B:	2-propanol

Column:	ACQUITY UPLC BEH C_{18} 2.1 x 150 mm
Method one:	22 minutes
Flow rate:	0.15 mL/min
Linear gradient:	10 to 90% B in 22 minutes
Method two:	12 minutes
Flow rate:	0.17 mL/min

Gradient

Time (min)	%B	Curve
0	11	6
7	37.5	6
7.01	90	11
12	90	11

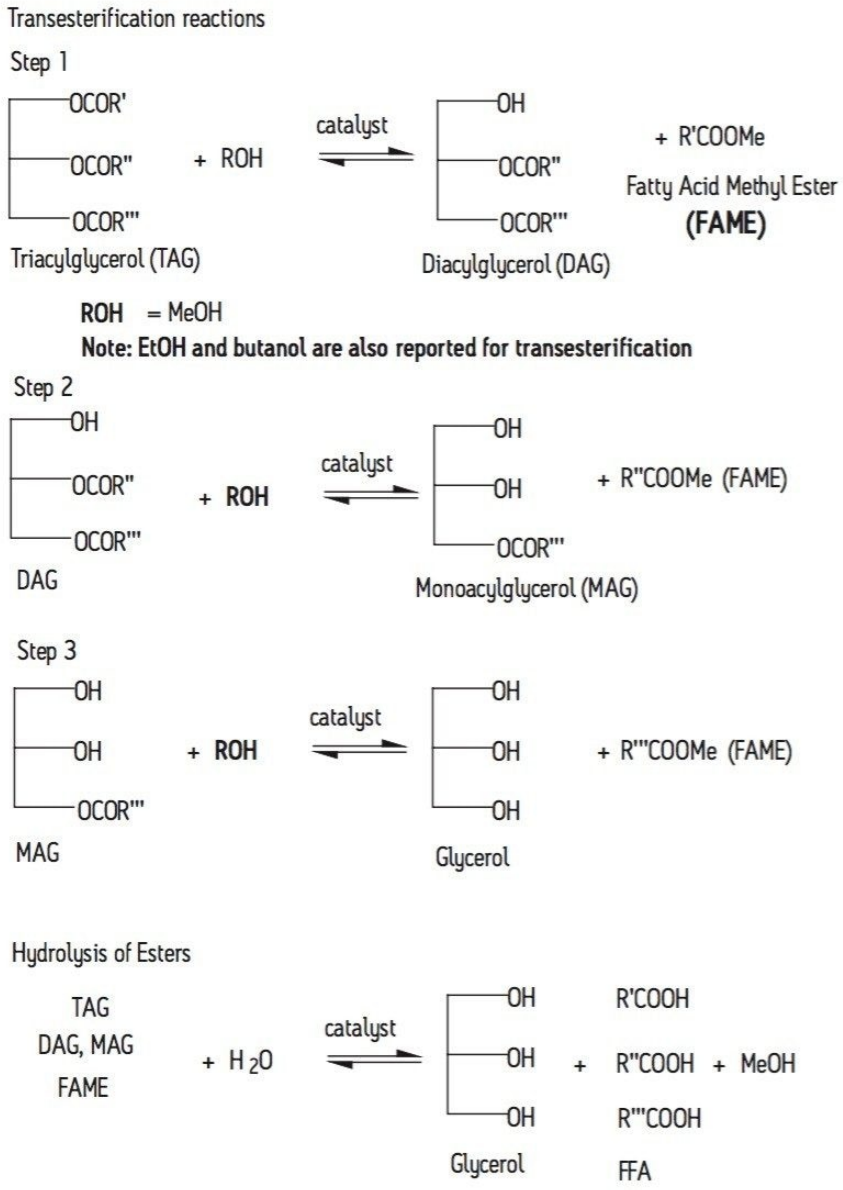
ELS Parameters

Gain:	500
Nebulizer:	Cooler
N2 gas pressure:	40 psi

Date rate:	20 pt/s
Drift tube temp.:	55 °C
Time constant:	0.1

Results and Discussion

Figure 1 illustrates the transesterification and hydrolysis reactions of esters, the critical chemical reactions involving biodiesel production using seed oils and animal fat as feedstock. Numerous efforts have been made to optimize the production processes such that the conversion of TAG to FAME is maximized while the contaminants in the final biodiesel product are minimized.[1,2,5,6] Since contaminants can arise during an improper production process or under poor storage conditions, a fast and reliable analytical method can be used at multiple stages to decrease the possibility for product failure.



We previously reported that the high-pressure fluidic modules of UPLC system enable high resolution and sensitive, fast separation of TAG components of seed oils with the ACQUITY UPLC small particle (1.7 µm) column technology using lower toxicity solvents, acetonitrile and 2-propanol as the mobile phase.[15,16] The UPLC method was applied to characterize the homemade biodiesel and the standard solution containing 18 biodiesel related analytical standards and soybean oil.

Table 1 lists the names and CAS numbers of the standards used in this study including glycerol (1), six FAME (4,

7, 10, 11, 13, and 15), six FFA (2, 5, 8, 9, 12, and 14) two MAG (3, 6), DAG (16), two TAG (17, 18) and soybean oil (19).

ID	Name	CAS No.	Peak Label
1	Glycerol	56-81-5	a
2	Linolenic acid	463-40-1	b
3	1-Linoleoyl-rac-glycerol	2277-28-3	b
4	Methyl linolenate	301-00-8	c
5	Linoleic acid	60-33-3	d
6	1-Oleoyl-rac-glycerol	111-03-5	d
7	Methyl linoleate	112-63-0	e
8	Oleic acid	112-80-1	f
9	Palmitic acid	57-10-3	f
10	Methyl oleate	112-62-9	g
11	Methyl palmitate	112-39-0	g
12	Stearic acid	57-11-4	h
13	Methyl stearate	112-61-8	i
14	Arachidic acid 17	506-30-9	j
15	Methyl arachidate 17	1120-28-1	k
16	1,3-dilinoleoyl-rac-glycerol	15818-46-9	l
17	1,2,3-trilinoleoylglycerol	537-40-6	m
18	Glyceryl trioleate	122-32-7	n
19	Soybean oil	8001-22-7	

Table 1. Biodiesel related standards and peak labeling.

Figures 2 and 3 show ELS and 210 nm PDA extracted chromatograms of the biodiesel and the standard solution obtained using a 22-minute linear gradient method with a 2.1 x 150 mm BEH C_{18} Column. Many well separated peaks are observed in the expanded chromatograms (Figure 2). Most of them are identified by comparing their retention times with the standards (Figure 3 and Table 1).

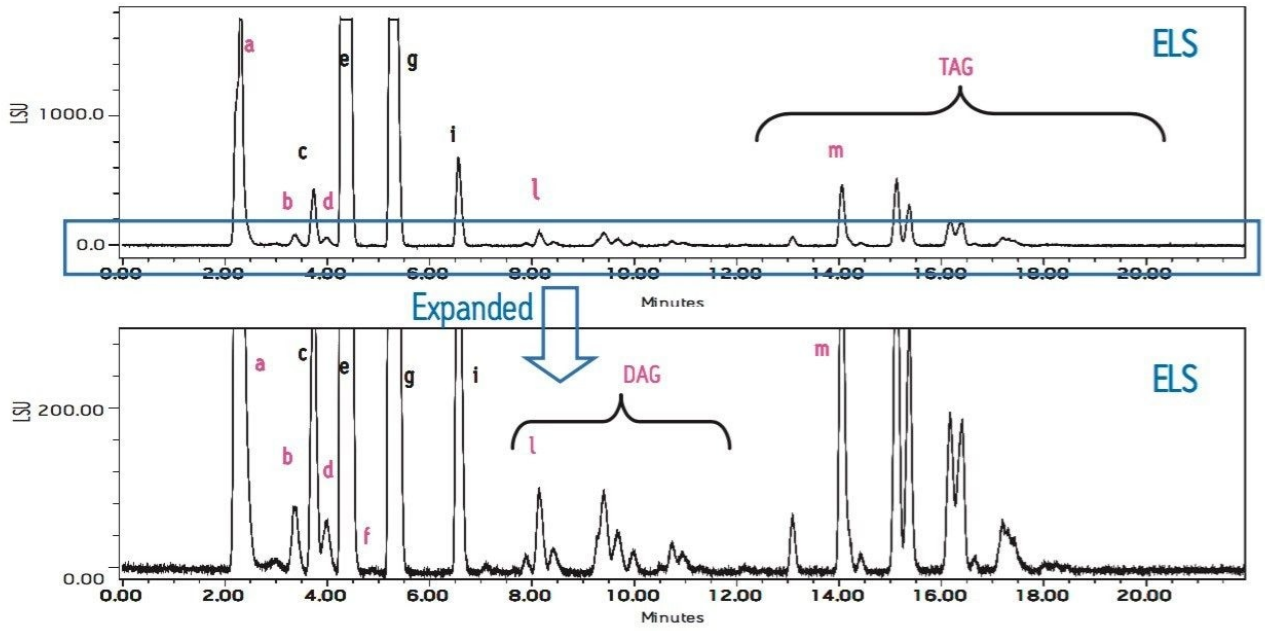


Figure 2a. ELS chromatogram of homemade biodiesel (12 mg/mL) made from soybean oil.

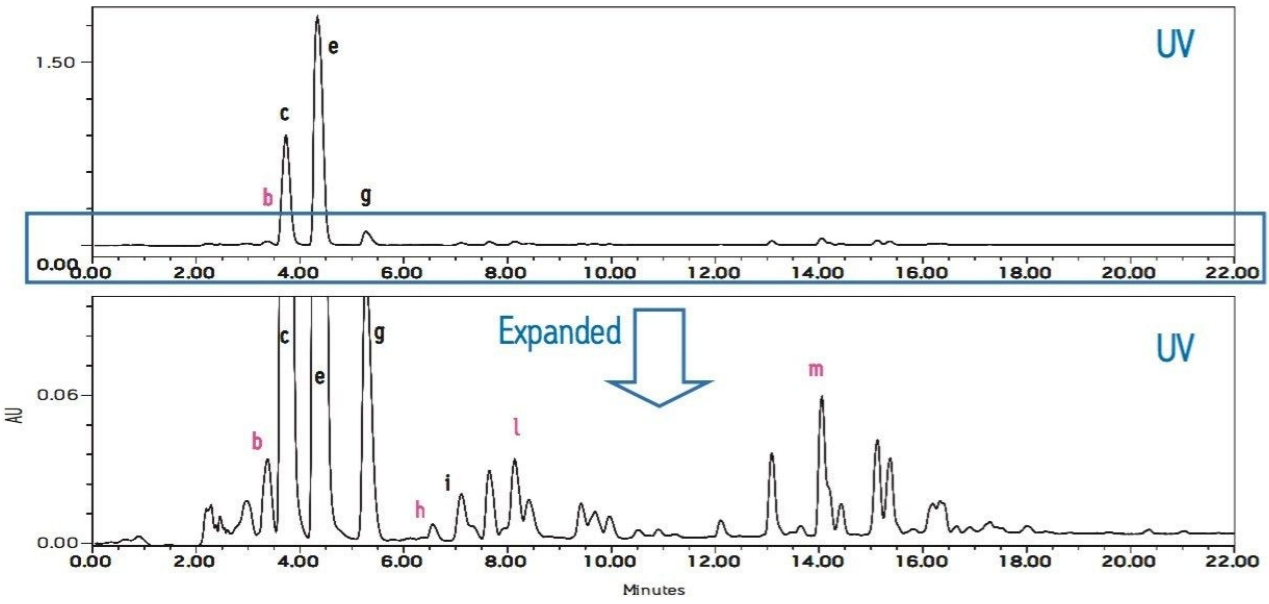


Figure 2b. UV (210 nm) chromatogram of homemade biodiesel (12 mg/mL) made from soybean oil.

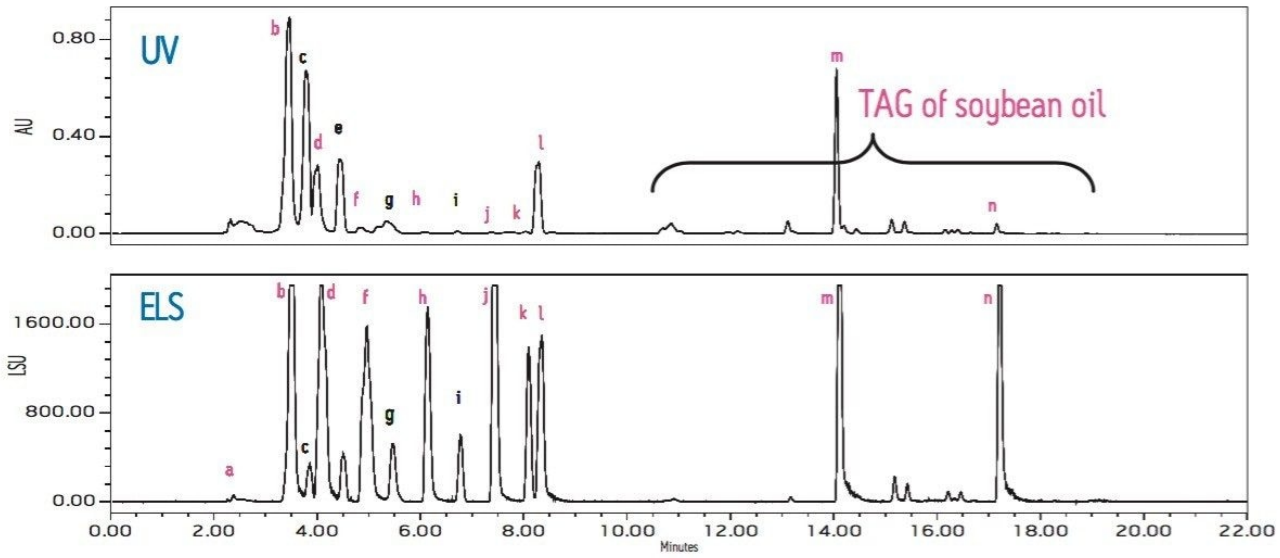


Figure 3. ELS and UV (210 nm) chromatograms of the standard solution using gradient method 1: soybean oil (0.7 mg/mL), FFA, FAME, MAG, DAG and TAG (0.5 mg/mL each).

Five FAME (4, 7, 10, 11, and 13), the final products of transesterification of soybean oil, are well separated from contaminants with retention times between 3.7 to 7 minutes as shown by the major peaks of the chromatogram: methyl linolenate in peak c, methyl linoleate in peak e, methyl oleate and methyl palmitate co-elute in peak g, methyl stearate in peak i.[17]

Glycerol (1), MAG (3 and 6), DAG (16), TAG (17), and the five FFA from unwanted hydrolysis reactions (2, 5, 8, 9, and 12) are also well separated: glycerol in peak a, 1-linoleoyl-rac-glycerol and linolenic acid co-elute in peak b, linoleic acid and 1-oleoyl-rac-glycerol in peak d, oleic acid and palmitic acid in peak f, stearic acid in peak 0 h, 1,3-dilinoleoyl-rac-glycerol in peak l, glyceryl trilinoleate in peak m.

The peaks with retention times longer than 12 minutes match well with that of TAG components of soybean oil. The peaks having retention time between 7 to 12 minutes are most likely reaction intermediates, DAG.

Comparison of retention time of standards shows that the separation is based on the number of alkyl chains, chain length and the number of double bonds (Figure 3 and Table 1). The analytes with fewer alkyl chains elute first. Among analytes with the same number of alkyl chains, those with a shorter chain length and a higher number of unsaturated bonds elute earlier. The methyl esters and FFA with unsaturated bonds have strong UV absorbance at 210 nm making it easy to observe a PDA extracted chromatogram.

These 210 nm extracted chromatograms can be used to precisely monitor the progress of transesterification reactions and unwanted hydrolysis reactions. Fortunately, low-UV absorbing components do have significant response under the ELS detection conditions (Figure 3). This illustrates the value of combining PDA and ELS detectors with an ACQUITY UPLC System for biodiesel analysis. With a single chromatographic run, the UV and non-UV absorbing components can be analyzed simultaneously.

Because all the critical components of biodiesel can be observed in a single experiment using this UPLC methodology, it is an efficient approach to monitor the transesterification status for process optimization. Ideally, in a batch-mode reaction process, TAG peaks of feedstock should decrease while the peaks of MAG, DAG, and FAME appear in the chromatograms. Meanwhile, the peak intensity of MAG and DAG should start to decrease when a substantial amount of TAG is consumed.

The peak intensity of FAME will continue increasing whereas the peaks of TAG, DAG, and MAG decrease and effectively “disappear” from the chromatograms. By plotting the peak intensity of UV chromatograms against reaction time, there is the potential to easily monitor the reaction kinetics. During the production processes, if any disturbance happens, it could be observed and confirmed by both PDA and ELS detection. In addition, the mobile phase used in the current experiment is compatible with mass spectrometry detectors, if needed, to obtain additional structural details.

In a well developed biodiesel production process, an engineer mainly relies on the data of the relative amount of FAME, FFA, and residual total TAG to make critical decisions. Under such circumstances, a method might be preferred to separate FAME from FFA and allow all the TAG components to elute together at the end of chromatogram since the residual total TAG can be easily and unambiguously quantified.

The 22-minute linear gradient UPLC method was optimized for the analysis of a subset of TAG, FAME, and FFA related compounds for resolution and throughput. Figures 4 and 5 show ELS and PDA extracted 210 nm chromatograms of the homemade biodiesel and the standard solution obtained using a 12-minute gradient method.

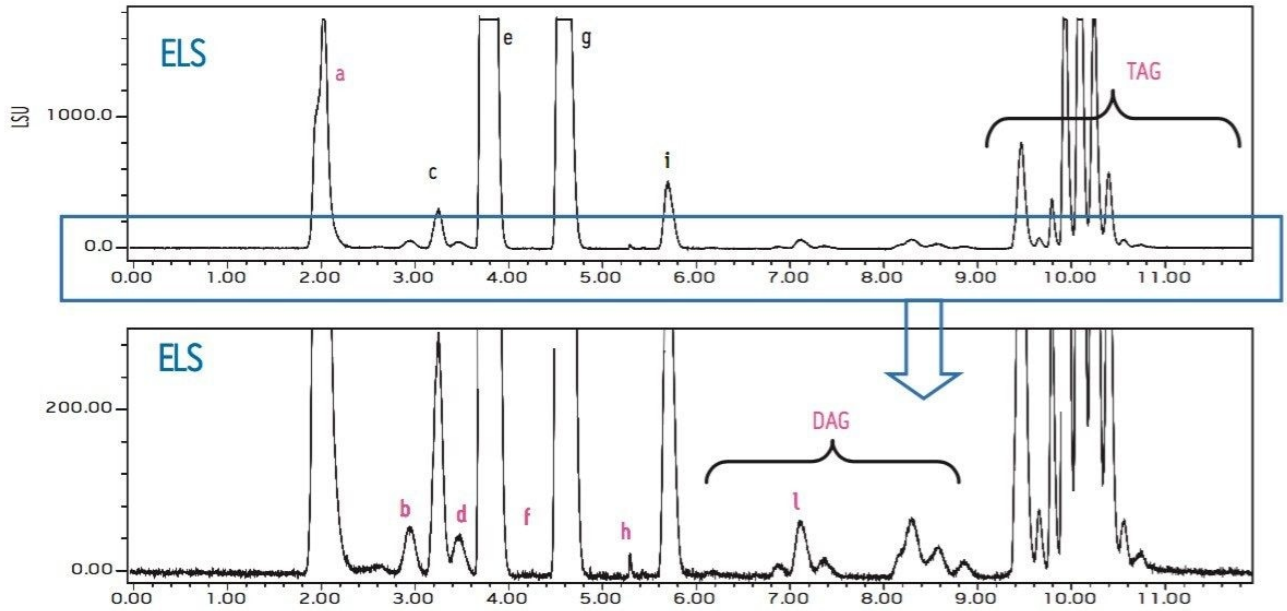


Figure 4a. ELS chromatogram of homemade biodiesel (12 mg/mL) made from soybean oil.

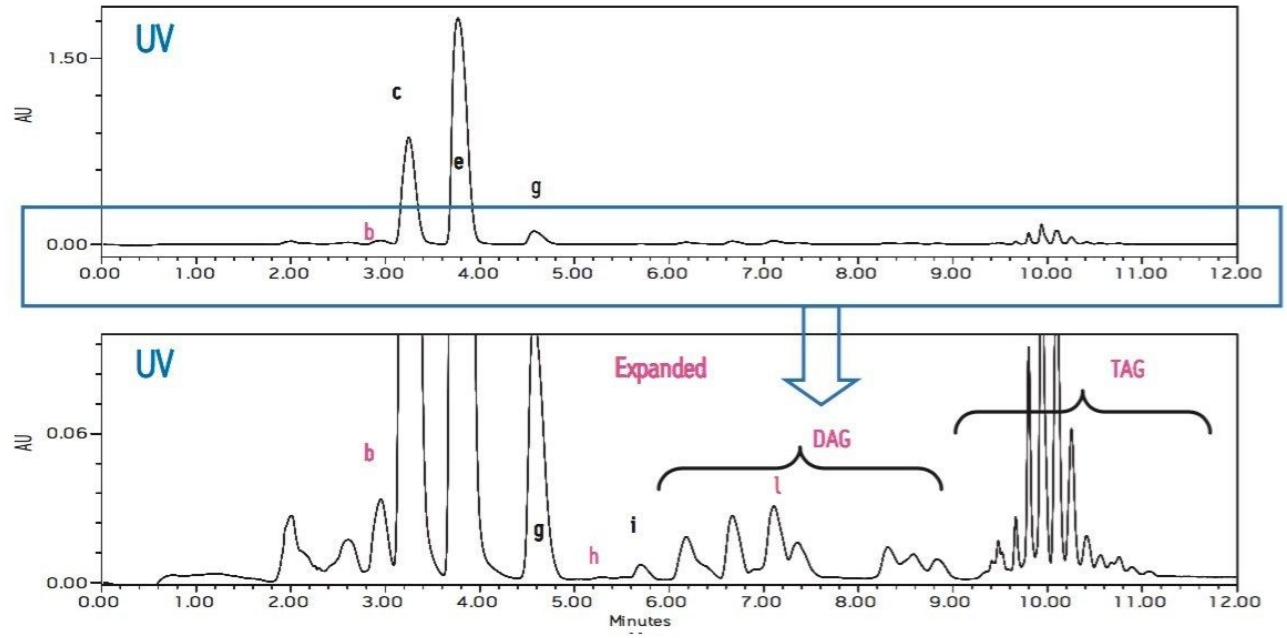


Figure 4b. UV (210 nm) chromatogram of homemade biodiesel (12 mg/mL) made from soybean oil.

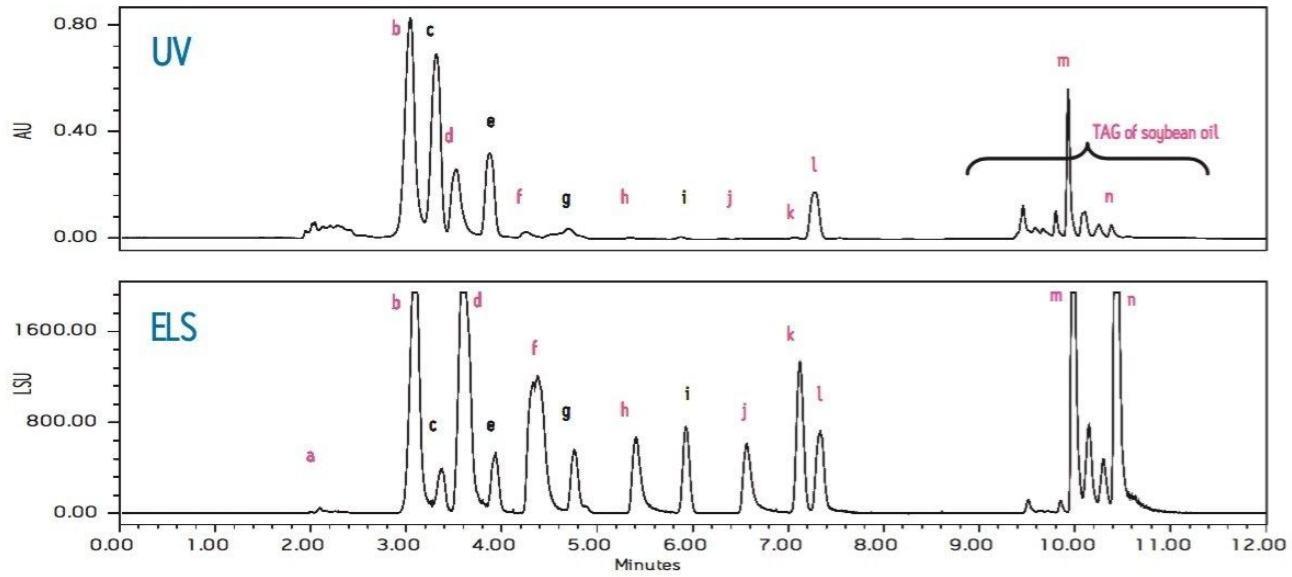


Figure 5. ELS and UV (210 nm) chromatograms of the standard solution using gradient method 2: soybean oil (0.7 mg/mL), FFA, FAME, MAG, DAG, and TAG (0.5 mg/mL each).

The first nine minutes of the 12-minute chromatogram are nearly identical to those of the 22-minute method shown in Figures 2 and 3 However, with the 12-minute method, all the TAG components elute in the 9.5 to 11 minutes range. The 12-minute gradient method can further increase the throughput of biodiesel product analysis.

Conclusion

The Waters ACQUITY UPLC System with PDA and ELS detectors is an ideal system for the analysis of biodiesel and organic contaminants. It enables rapid, sensitive, high resolution separations during process monitoring, and of final product in a single experiment. The separation is several times faster than conventional HPLC without using toxic halogenated solvents.

An additional value for applying UPLC technology for biodiesel analyses is reduced solvent consumption and hazardous solvent waste disposal, resulting in cost and safety benefits. By employing the complementary

detection of UV and evaporative light scattering, more information per chromatographic run was obtained, thus dramatically increasing productivity. Other industries such as agricultural seed development, medical applications, food, cosmetic, and personal care with an interest in seed oils and FFA could also benefit from this methodology.[15,16]

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17. Typical soybean oil consists of 11% palmitic acid, 4% stearic acid, 24% oleic acid, 54% linoleic acid, and 7% linolenic acid.

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720002155, April 2007