One Methodology For FFA, Fame and Tag Analysis In Biodiesel Using UltraPerformance LC and Evaporative Light Scattering and Photodiode Array Detection

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Introduction

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. To minimize this, 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. Gas chromatography (GC) and high performance liquid chromatography (HPLC) are common analytical techniques for this analysis. However, these techniques consume valuable personnel and instrumentation time. Also lab workers are often exposed to carcinogenic, halogenated solvents to perform needed analyses.

Figure 1. Three-step reaction of transesterification and hydrolysis of ester

The Waters® ACQUITY UltraPerformance LC® (UPLC®)/ Photodiode Array (PDA)/Evaporative Light Scattering Detector (ELSD) system provides one methodology with lower toxicity solvents, acetonitrile and 2-propanol. The 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. Numerous efforts in government, corporate and academic labs are directed toward optimizing production processes such that the conversion of TAG to FAME is maximized while the contaminants in final biodiesel product are minimized. Since contaminants can arise during improper production or under poor storage conditions, a fast and reliable analytical method used at multiple stages can decrease the possibility of product failure. The ability to quickly and reliably analyze these critical components can facilitate the monitoring of 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 a kitchen biodiesel method based on a supermarket brand soybean oil, reagent grade methanol and sodium hydroxide. A portion of biodiesel was diluted with 2-propanol (IPA) and filtered through a 0.45µm PVDF syringe filter to make a 12mg/mL solution for UPLC analysis. Concentrations of 0.5mg/mL and 0.7mg/mL, respectively, of standards and soybean oil in IPA were used for analysis.

UPLC® System and Operation Conditions

LC System:	ACQUITY UPLC System/ACQUITY PDA/ELSD
Software:	Empower™ 2 Chromatography Software (build 2154)
Column T:	30 °C
Injection:	2 µL
Mobile Phase:	A: Acetonitrile; B: 2-propanol
Column:	ACQUITY UPLC BEH C18 2.1x 150 mm
Method 1:	22 minutes, 0.15mL/min, Linear Gradient
Method 2:	12 minutes, 0.17mL/min, Non-linear Gradient

Results And Discussion

The utility of the UPLC methodology is illustrated with a 22 minute run for increased component resolution across the entire time frame or a 12-minute run with lower resolution only in the TAG region. The samples were homemade biodiesel and standards of glycerol (1), six FAME (4, 7, 10, 11, 13 & 15), six FFA (2, 5, 8, 9, 12, & 14) two MAG (3, 6), DAG (16), two TAG (17, 18) and soybean oil (19).

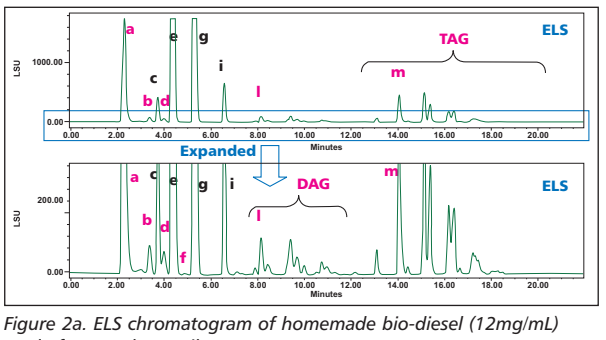

Figure 2a. ELS chromatogram of homemade bio-diesel (12mg/mL) made from soybean oil.

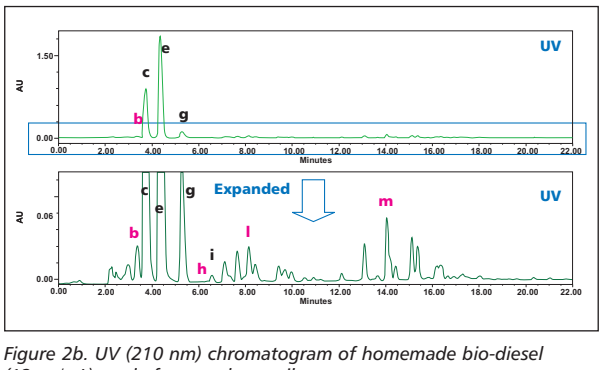

Figure 2b. UV (210 nm) chromatogram of homemade bio-diesel (12mg/mL) made from soybean oil.

The biodiesel chromatograms represented by *Figure 2* illustrate the 22-minute UPLC method for increased component resolution. By comparing peak retention times to the standards *(Figure 3 & Table 1)* five FAME (4, 7, 10, 11, & 13) between 3.7 to 7 minutes are assigned: 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. Glycerol (1), MAG (3 & 6), DAG (16), TAG (17) and the five FFA from unwanted hydrolysis reactions (2, 5, 8, 9, & 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 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 the TAG components of soybean oil. The peaks having retention time between 7 to 12 minutes are most likely reaction intermediates, DAG.

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 labels

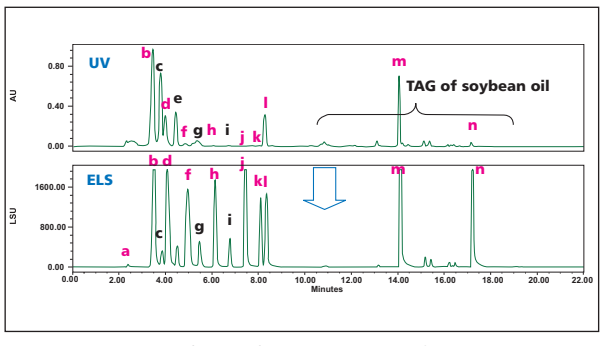

Figure 3. ELS and UV (210 nm) chromatograms of the standard solution using gradient method 1:soybean oil (0.7mg/mL), FFA, FAME, MAG, DAG and TAG (0.5mg/mL each)

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 & 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.

In a well-developed biodiesel production process, obtaining the relative amount of FAME, FFA and residual total TAG could be sufficient to make critical process decisions. In this case the 12-minute method illustrated in Figure 4 with the homemade biodiesel solution compresses the TAG region somewhat but maintains resolution in the FAME and FFA region. It's easy to envision increased throughput of biodiesel process & product analyses with this 12-minute run.

Conclusion

The Waters ACQUITY UPLC system with PDA and ELS detectors is ideal for the analysis of biodiesel and organic contaminants. It enables rapid, sensitive, high resolution separations for process monitoring and final esterified product.

The separation is faster than conventional techniques, derivatization is unnecessary and toxic halogenated solvents are not used. An additional value from using the UPLC system is reduced solvent consumption and disposal, resulting in lower cost and increased safety benefits.

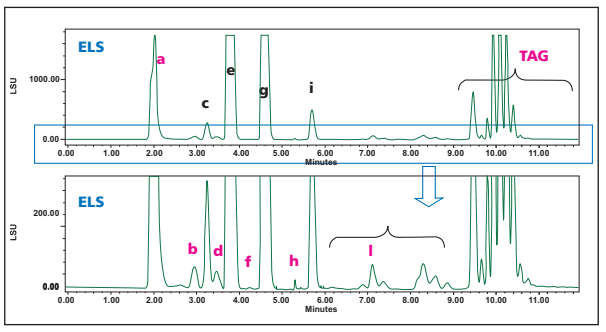

Figure 4a. ELS chromatogram of homemade bio-diesel (12mg/mL) made from soybean oil.

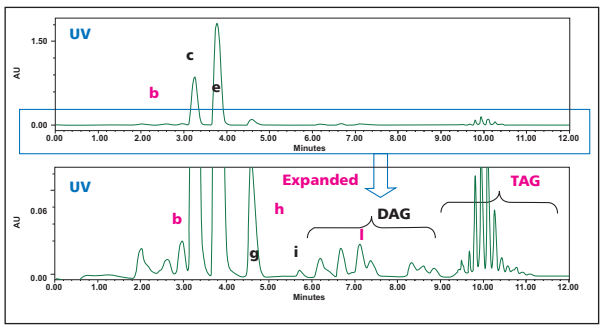

Figure 4b. UV (210 nm) chromatogram of homemade bio-diesel (12mg/mL) made from soybean oil.

Petrobras Adopts LabVIEW to Improve Oil and Gas Exploration

Graphical Programming Approach Saves Company up to $2 Million. Petrobras, one of Latin America's largest oil and gas companies, has selected **National Instruments** (USA), LabVIEW software and data acquisition hardware to develop a mud-gas separator for underbalanced drilling (UBD) applications. Using NI tools, Petrobras engineers have developed a high-throughput UBD application capable of reducing the time it takes for a well to become fully productive as well as eliminating the need for costly fracturing after the well is completed.

By deploying UBD technology based on LabVIEW, Petrobras has realised savings between $500,000 and $2 million, depending on the size of the well and the cost of the potential fracturing job, said Manoel Feliciano da Silva, Equipment Engineer at Petrobras. "This method, made more efficient through the use of NI products, gives Petrobras the ability to reach full production faster and with minimal impact on the formation."

UBD is a drilling technique that reduces the hydrostatic pressure of the drilling fluid, causing the pressure in the wellbore to be less than the formation pressure. As the well is being drilled, formation fluid flows into the wellbore and up to the surface. This approach provides significant financial and operational improvements over traditional drilling techniques. In traditional drilling, pressure often causes formation damage and results in a delay before the formation reaches full production or forces companies to perform fracturing jobs on the well. In addition to being more financially efficient, the new system is intrinsically safer and easier to use than traditional methods.

The mud-gas separator Petrobras developed delivers a compact separation system capable of handling the dynamic gas and liquid flow rates generated during drilling with compressible fluids, and it responds quickly to variations in the pressure or flow rates from injected or produced variations in the dynamic systems. LabVIEW provides connectivity to the drilling control programmable logic controller (PLC) and connectivity to remote systems. LabVIEW also delivers the flexibility to connect to existing PLCs; perform high-speed measurements; and provide the required server, client and playback modes.

The LabVIEW system exceeds Petrobras design requirements, providing real-time measurements and trending to equip operators with better data for decision making and improved safety along the drilling operations. The equipment for land operation was tested and approved during full-scale experiments and is currently being used on four oil wells drilled at the Estreito Field located in the Rio Grande do Norte state.

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High Quality Gas Generators for Gas Chromatographs

Peak Scientific (UK) offers a range of Gas Generators specifically designed to supply Hydrogen, UHP Nitrogen and Zero Air to Gas Chromatographs. GCs can typically be operated with Helium, Nitrogen, Argon, Nitrogen and Air, but which gas to use is usually determined by the detector being used. Safety and availability can also influence the selection of gases used for your analysis, e.g. high purity Helium can be difficult to obtain in some countries and gas bottles and cylinders pose a potential Health & Safety Hazard. The purity of the gases required is frequently determined by the detector, though the level of sensitivity needed can also play a significant role.

The gas flow rate affects the analysis in the same way that temperature does and the higher the flow rate the faster your analysis, but the lower the separation between the analytes. Selecting the flow rate is therefore the same compromise between the level of separation and length of analysis as selecting the column temperature. All this has been considered when Peak Scientific developed their products. Their range of Hydrogen, Nitrogen and Zero Air Generators offer a safe and cost effective solution to your gas requirements whilst providing you with the purities and pressures required to operate your GCs. With different flow rates available the gas generators will provide you with a suitable supply of laboratory gas for your specific application and all of Peak Scientific's generators have been tried, tested & approved by leading instrument manufacturers.

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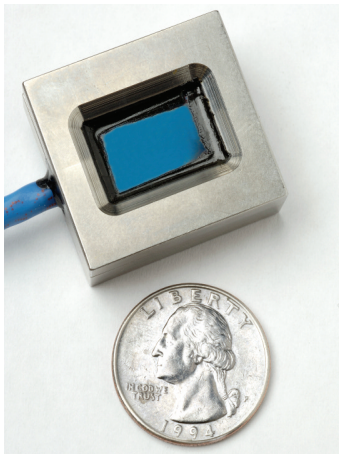
The Smart Solution to a Wide Range of Viscosity Measurement Applications



Vectron Inc. (USA) has introduced the new ViSmart™ solid-state viscometer designed to provide instantaneous sample and/or continuous, real-time, in-process viscosity measurements for process control in a robust cost-effective package weighing just 4 oz. The ViSmart™, with integrated temperature measurement capability uses robust and reliable semiconductor technology and has no moving parts packaged in a very portable size. It is unaffected by vibration or flow conditions and does not need field calibration. The ViSmart™ integrates to the eCup™ handheld reader or the ViscNet™ control hub where on board-electronics then transmit viscosity and temperature readings in real-time using standard communications protocols to provide a continuous audit trail to help control operating costs and maintain quality standards. The ViSmart™ at a size of 1.3in x 1.1in x 0.4in is hermetically sealed for complete immersion in fluid stream for process control or can be used in lab environments. The ViSmart™ measures viscosity of fluid from 10 to 10,000 centipoise and rated for temperatures up to 135?C in the standard package, with custom options and configurations available for specific industry applications and process environments. The ViSmart™ is designed for challenging manufacturing environments to provide temperature and viscosity measurement ranging from oil and lubricants to coatings and wide variety of chemicals and processing fluids.

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