[APPLICATION NOTE]

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Optimized Method for Analysis of Commercial and Prepared Biodiesel using UltraPerformance Convergence Chromatography (UPC^2)

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APPLICATION BENEFITS

- No analyte pre-column derivatization thus eliminating artifact formation
- Complete separation of all components in less than 12 minutes
- No thermal degradation of the analytes

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ACQUITY UPC^2 System with an ACQUITY UPC^2 ELS Detector

ACQUITY UPC2 HSS C_{18} SB Column

KEY WORDS

SFC, convergence chromatography (CC), UltraPerformance Convergence Chromatography, UPC,^{2} triacylglycerols, TAG, glycerol, biodiesel, biofuel, fatty acid alkyl esters, FAMEs, FAEEs, ELSD

INTRODUCTION

Commercial biodiesel is usually obtained by trans-esterification of vegetable oil using methanol or ethanol as a reactant. The resulting products are fatty acid methyl esters (FAMEs) or fatty acid ethyl esters (FAEEs). As an energy source, biodiesel should meet certain criteria. For example, the level of impurities such as triacylglycerol, diacylglycerol, monoacylglycerol, and free glycerol should be 0.2% to 0.8% for acylglycerols and 0.02% for glycerol. These impurities vary in polarity, solubility, and volatility.

The standard procedure for analysis of biodiesel impurities usually involves gas chromatography (GC) , but one must resort to either high temperature (370 °C) or pre-derivatization for conversion to more volatile products in order to be successful. High temperature GC usually requires harsh conditions, causing degradation and/or rearrangement of certain analytes. Furthermore, derivatization usually involves many steps to prepare the sample which can be time consuming.

UltraPerformance Convergence Chromatography™ (UPC$^{2®}$) is a novel technology that applies the performance advantages of UPLC® to supercritical fluid chromatography (SFC). Combining the use of supercritical CO_2 with sub-2-µm particle columns, UPC^2 represents an analysis technique that is orthogonal to reversed-phase liquid chromatography (RPLC) and can be used to solve many troublesome separations that challenge conventional LC or GC analyses.

Previously, a UPC^2 method that uses an ACQUITY UPC$^{2™}$ HSS C_{18} SB Column and evaporative light scattering (ELS) detection was developed for analysis of biodiesel and impurities in spiked model mixtures.[1] The method to analyze both a series of biodiesels prepared in-house from tobacco seed oil[2] and a commercially-available B100 biodiesel is described here. In addition, to obtain a cleaner biodiesel, a simple purification method employing gravity flow with a prepared glass column packed with bare silica was applied to an in-house synthesized biodiesel using hexane and ethanol as tandem eluents.

EXPERIMENTAL

Sample preparation

Octadecyl glycerol standards were purchased from Sigma-Aldrich (St. Louis, MO). Biodiesel B100 was obtained from a commercial source. Synthetic biodiesel derived from tobacco seed oil (R.J. Reynolds Tobacco Co., Winston-Salem, NC) was prepared in- house via trans-esterification in ethanol using three different batches of oil. Biodiesel purification was performed using column chromatography on bare silica via gravity flow. Silica Gel (60Å, 200-420 mesh) was also purchased from Sigma Aldrich.

Method conditions

System:	ACQUITY UPC^2
Column:	ACQUITY UPC^2 HSS C_{18} SB, 1.8 µm, 3.0 x 150 mm
Sample:	5% sample in DCM/MeOH
ABPR:	1500 psi
Column temp.:	25 °C
Injection volume:	2-8 µL
Sample solvent:	DCM/MeOH (50/50)
Flow rate:	1-2 mL/min
Mobile phase A:	Compressed CO_2
Mobile phase B:	Acetonitrile/methanol (90/10)
Make up solvent:	IPA
Make up flow rate:	0.2 mL/min
Gradient:	See Figure 1 for different methods
Detectors:	ACQUITY UPC^2 PDA 210 nm, Ref. 400 to 500 nm
ACQUITY UPC^2 ELS:	Nebulizer: Cooling, Drift Tube: 50 °C, Gas Pressure: 40 psi, Gain: 10, Make up flow was added to UPC^2 column effluent, Split for BPR and ELSD was 1:3

RESULTS AND DISCUSSION

Figure 1 shows the separation of a mixture of C_{18} triacylglycerol, diacylglycerol, and monoacylglycerol, plus free glycerol spiked into model biodiesel, which was composed of a mixture of FAEEs. Different gradient elution profiles and flow rates were employed in order to achieve faster analysis with minimal loss in resolution of all impurities. Figure 1 - Method A was used originally to develop the separation in a previous application note.[1] The analysis time for this method was 17 minutes. However, in order to reduce the analysis time, the initial flow rate as well as the starting and ending modifier percentages, were adjusted to afford separation of all compounds in less than 10 minutes (Figure 1 - Method B). Finally, the gradient time was further reduced in order to achieve an analysis time of less than 5 minutes with still adequate resolution of all impurities. Method C is, therefore, suitable for analysis of biodiesel with a lesser amount of impurities. However, due to the high concentration of synthetic biodiesel derived from tobacco seed oil in the sample, as well as elution of various triacylglycerols close to the tail of the biodiesel peak which might interfere with quantification, the second method (Method B) was used for all further analyses. A faster separation with little loss in analyte resolution would translate into higher sample throughput for monitoring product quality.

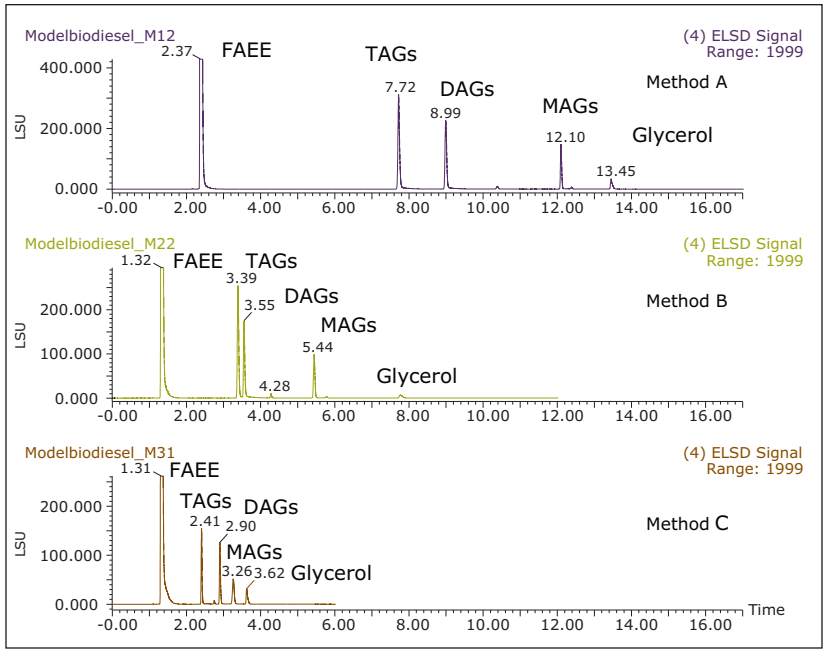

Figure 1. UPC^2 Single Injection of a mixture of model biodiesel, glycerol, and C_{18} acylglycerols with different gradients.

Gradient elution for Method A: T= 0 min, 98:2 CO_2/Modifier, T=10 min, 80:20, T=12 min, 50:50, T=15 min, 50:50, T=15.5 min, 98:2, T=17 min, 98:2, Flow: 1.0 mL/min., Oven temp.: 25 °C, Modifier 90:10 CH_3CN/MeOH.

Gradient Elution for Method B: T=0 min, 90:10 CO_2/Modifier, T=10 min, 50:50, T=11 min, 50:50, T=11.1 min, 90:10, T=12 min, 90:10, Flow: 1.2 mL/min., Oven temp.: 25 °C, Modifier 90:10 CH_3CN/MeOH.

Gradient Elution for Method C: T=0 min, 90:10 CO_2/Modifier, T=2 min, 50:50, T=6 min, 50:50, T=6.1 min, 90:10, Flow: 1.2 mL/min., Oven temp.: 25 °C, Modifier 90:10 CH_3CN/MeOH.

Method B was therefore used to determine the purity of synthetic biodiesels prepared in-house derived from three tobacco seed oil lots (Figure 2). As can be observed, all three biodiesel samples showed the presence of multiple impurities (retention time window of 2 to 4 min), as well as monoacylglycerols at a retention time of 6.2 min. Total impurities were highest in batch 2 and lowest in batch 3. For comparison, a commercial B100 biodiesel sample was obtained and analyzed (Figure 3A). As can be observed, monoacylglycerols were also detected in the commercial B100 biodiesel based on retention time. Other impurities were observed in the 2 to 4 minute retention window, but they were not identified. Figure 3B shows the separation of model biodiesel (i.e., pre-mixed FAEEs) spiked with different known impurities for comparison.

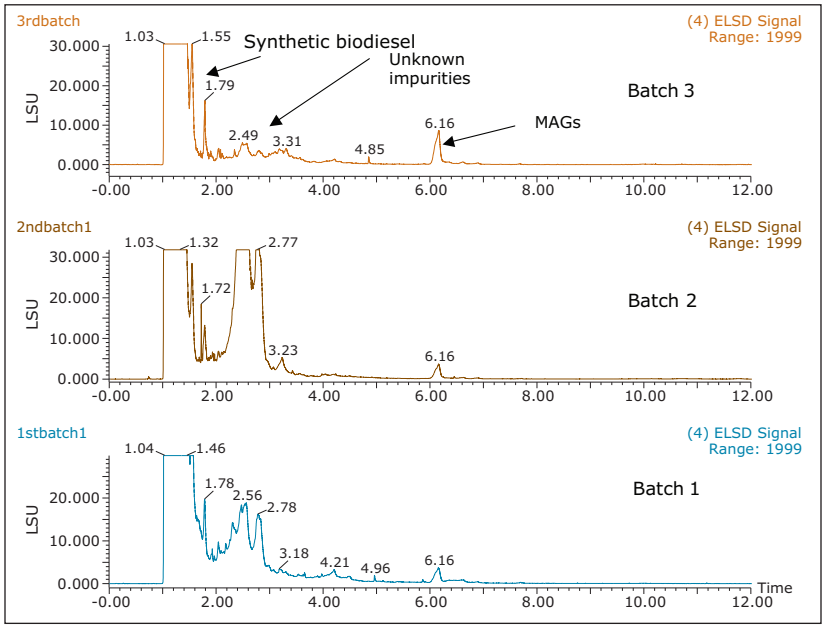

Figure 2. UPC² chromatograms of three different batches of biodiesel derived from tobacco seed oil.

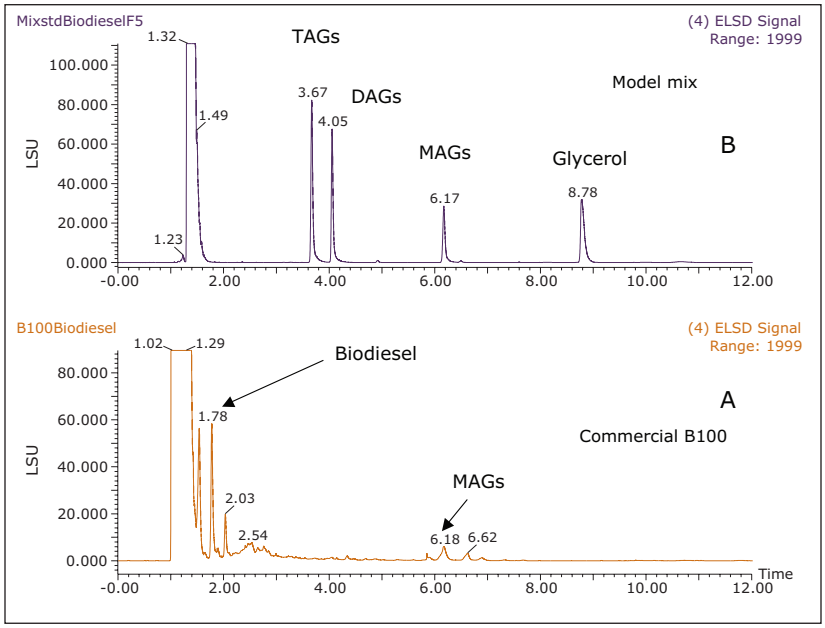

Figure 3. Injection of commercial biodiesel B100 and model biodiesel mix spiked with different impurities.

It is important for manufacturers and industries to have pure biodiesel with no glycerol or acyglycerols as long as their removal is commercially viable. Therefore, it is essential to efficiently remove these impurities. Currently, the industry makes use of the density difference to separate the bulk of the glycerol impurity. In this application, we have developed a method to remove all impurities, including glycerol, from synthetic biodiesel via a simple, two-step column chromatographic process. More specifically, biodiesel derived from tobacco seed oil and the associated impurities were passed through a bare silica column and eluted via gravity first using hexane and then using ethanol.

Each fraction was then evaporated and the resulting sample was dissolved in MeOH/DCM (1:1). Analysis of the fractions was performed using the UPC^2 method described in Figure 1B. Figure 4 shows the separation of the synthetic biodiesel before purification, after purification with hexane as the eluting solvent, and after purification with ethanol as the eluting solvent. As can be observed, nearly pure synthetic biodiesel was obtained via the hexane fraction. Peaks eluting in the same retention window as monoacylgycerols were separated and detected in the ethanol fraction of each biodiesel sample. This purification process should be suitable for industries that are required to have pure biodiesel (FAEE or FAME). Using Method C, UPC^2 can easily be used to show the purity of sample in less than 5 minutes. The ability to inject hexane and ethanol fractions directly onto UPC^2 allows for rapid assessment of the purity of biodiesel.

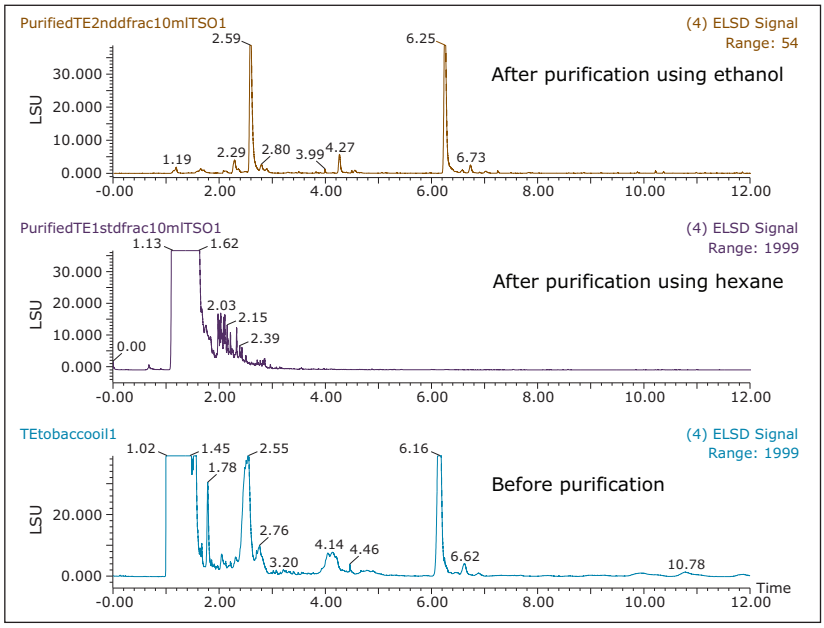

Figure 3. Injection of commercial biodiesel B100 and model biodiesel mix spiked with different impurities.

CONCLUSIONS

A single ACQUITY UPC^2 HSS C_{18} SB Column packed with 1.8-µm particles was successfully used to separate a mixture of glycerol, acylglycerols, and FAEEs (*i.e.*, model biodiesel). A gradient of CO_2 and acetonitrile/methanol served as the mobile phase and detection was performed by ELS detection. The optimized method provided fast separation and detection of all impurities in biodiesel without employment of sample derivatization or sample preparation. The method was used to detect impurities in three different synthetic biodiesels derived from tobacco seed oil and a commercial biodiesel. A simple, two-step, column chromatographic method using bare silica afforded nearly pure biodiesel fractions. This level of biodiesel purity can be determined using UPC^2 in less than five minutes.

References

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2. Mukhtar H, Ullah H, Mukhtar A. "Fatty acid composition of tobacco seed oil and synthesis of alkyd resin." Chin. *J. Chem.*, 25 (2007) 705.

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