# A Reversed-phase HPLC Method Using Evaporative Light Scattering Detection (ELSD) for Monitoring the Reaction and Quality of Biodiesel Fuels

A reversed-phase HPLC method using ELSD was developed for the detection and quantification of mono-, di-, and triacylglycerols in the synthesis of biodiesel from vegetable oils. The concentrations of these components are the key parameters in monitoring the transesterification reaction by which biodiesel is produced and for assessing biodiesel fuel quality.

Vegetables oils, such as soybean oil, rapeseed oil, corn oil, palm oil and others as well as animal fats and recycled greases are the major sources of biodiesel. Regardless of the feedstock, transesterification reactions are carried out to produce biodiesel. The transesterification reaction of triacylglycerols (TAGs) in oils is usually done by reacting the TAGs with methanol in the presence of a basic catalyst yielding the fatty acid methyl ester (FAME). During the transesterification process, intermediate glycerols such as monoacylglycerols (MAGs) and diacylglycerols (DAGs) are formed which can remain in the final biodiesel product. Besides these MAGs and DAGs, unreacted TAGs can also be present and contaminate the final product. The contaminants can lead to severe engine problems. Therefore, it is very important to have an analytical method to monitor the transesterification reaction as well as to be able to quantify the contaminants to very low levels.

## Experiment:

Agilent 1100 HPLC System

Alltech® Model 3300 ELSD

**Column:** Alltech® Alltima™ HP C18 Hi-Load, 5µm, 250 x 4.6mm

**Mobile Phase: A:** Acetonitrile **B:** Dichloromethane

**Gradient:**

| **Time:** | 0 | 5 | 30 | 32 | 35 |
|---|---|---|---|---|---|
| **%B:** | 0 | 15 | 70 | 70 | 0 |

**Flow Rate:** 1.0mL/min

## Conclusion

The above gradient method with the Alltech® Alltima™ HP C18 Hi-Load column and the Alltech® 3300 ELSD enables the separation and low-level detection of mono-, di- and triacylglycerols in biodiesel. This method is useful in analyzing starting material, monitoring the transesterification reaction and low level of impurities quantification (down to 0.008%). **Figure 1** and **Figure 2** show chromatograms of biodiesel derived from soybean oil. Figure 1 shows biodiesel that was found to be within specifications (less than 0.24% total and free glycerol) with low levels of mono-, di- and triacylglycerols. Figure 2 shows biodiesel that was found to be out of specification with high levels of mono-, di- and triacylglycerols.

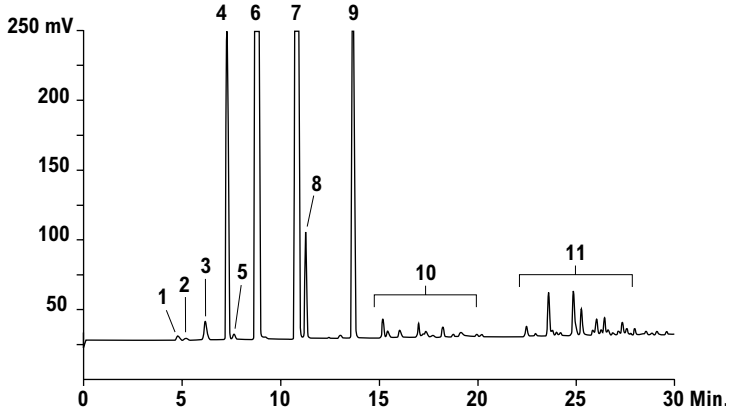


**Figure 1:** Chromatogram of Biodiesel in specification with ASTM method and EN14105 requirements for mono-, di-, and triacylglycerol in biodiesel (less then 0.24% total glycerol and free glycerol). 1. MAG, 2. MAG, 3. MAG, 4. FAME, 5. MAG, 6. FAME, 7. FAME, 8. MAG, 9. FAME, 10. DAG's, 11. TAG's

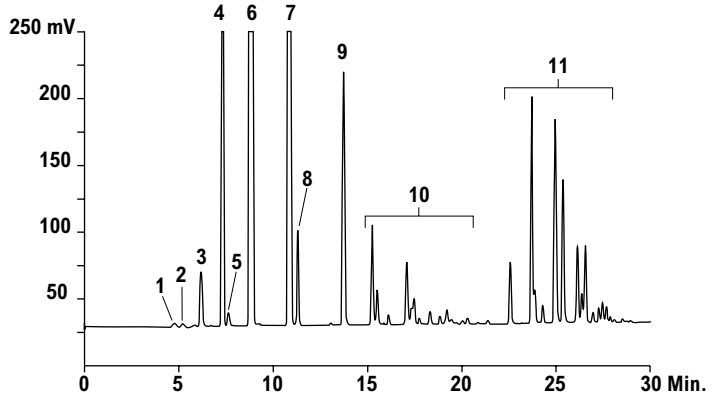


**Figure 2:** Chromatogram of Biodiesel out of specification with ASTM method and EN14105 requirements for mono-, di-, and triacylglycerol in biodiesel (greater then 0.24% total glycerol and free glycerol) Peak 1. MAG, 2. MAG, 3. MAG, 4. FAME, 5. MAG, 6. FAME, 7. FAME, 8. MAG, 9. FAME, 10. DAG's, 11. TAG's

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