Application Note: 51853

# Determination of Free Glycerol in Biodiesel with the Evolution Array UV-Visible Spectrophotometer

*Nicole Kreuziger Keppy, Gordon Bain, Ph.D., Michael W. Allen, Ph.D., Thermo Fisher Scientific, Madison, WI, USA*

**Key Words**

- ASTM Standards
- Biodiesel
- EN Standards
- Free Glycerol
- Transesterification
- UV-Visible Spectroscopy

## Introduction

The consumption of biodiesel as an alternative fuel source is on the rise. Biodiesel is used in its pure form or added to fuel blends to power automobiles and heat homes. Biodiesel is typically produced from vegetable oils and animal fats by adding an alcohol, usually methanol, to triglycerides in a transesterification reaction as shown in Figure 1 below. The product of the transesterification reaction is the biodiesel fuel itself in the form of fatty acid methyl esters (FAME) and a glycerol by-product.[1] Free glycerol affects the quality of biodiesel and its content is regulated by industry standards. In this experiment, the amount of free glycerol in biodiesel is measured with a Thermo Scientific Evolution Array UV-Visible spectrophotometer for conformance to American (ASTM)[2] and European (EN)[3] standards for free glycerol using the Quantification Mode of the Thermo Scientific VISION*collect* software.

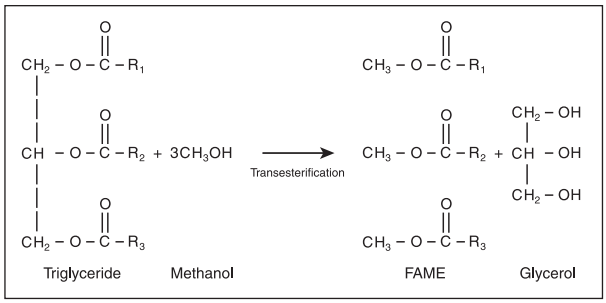


Figure 1: Generation of biodiesel by transesterification

## Experiment

The amount of free glycerol in biodiesel can be measured with a UV-Visible spectrophotometer using a two-step reaction process. This results in the formation of a yellow complex proportional to the amount of free glycerol in the sample. The sample is first treated with sodium periodate. Sodium periodate reacts with free glycerol in the sample to generate formaldehyde. Reaction between this formaldehyde and acetyl acetone produces the yellow complex, 3,5-diacetyl-1,4-dihydrolutidine (Figure 2). This yellow compound exhibits a maximum absorbance peak at 410 nm, where its concentration in the sample is measured.

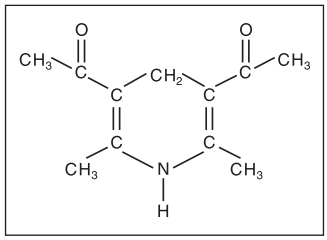

Figure 2: Chemical structure of 3,5-diacetyl-1,4-dihyrdrolutidine

A solvent solution containing a 1:1 ratio of deionized water and 95% ethanol, and a reference solution of 0.036 mg/mL glycerol in solvent was prepared. A series of six glycerol reference standards was then prepared from these solutions, as shown in Table 1. A pretreated biodiesel sample[4] was mixed 1:4 with solvent to get 2 mL of working sample solution.

| Standard | Glycerol Reference Solution (mL) | Solvent Solution (mL) | Final Concentration of Glycerol (mg/kg) |
|---|---|---|---|
| 1 | 0.00 | 2.00 | 0.00 |
| 2 | 0.25 | 1.75 | 3.75 |
| 3 | 0.50 | 1.50 | 7.50 |
| 4 | 0.75 | 1.25 | 11.25 |
| 5 | 1.00 | 1.00 | 15.00 |
| 6 | 1.25 | 0.75 | 18.75 |

Table 1: Glycerol standard preparation and absorpbance results

Each working standard and the sample were treated with 1.2 mL of a 10 mM sodium periodate solution and shaken for 30 seconds. Each solution was then treated with 1.2 mL of a 0.2 M acetylacetone solution, placed in a water bath at 70 °C for 1 minute and stirred manually. The solutions were immediately placed in cold water to stop the reaction. Standards and samples were measured using the method parameters shown in Figure 3. Standard 1 is a control sample and also used as the blank.

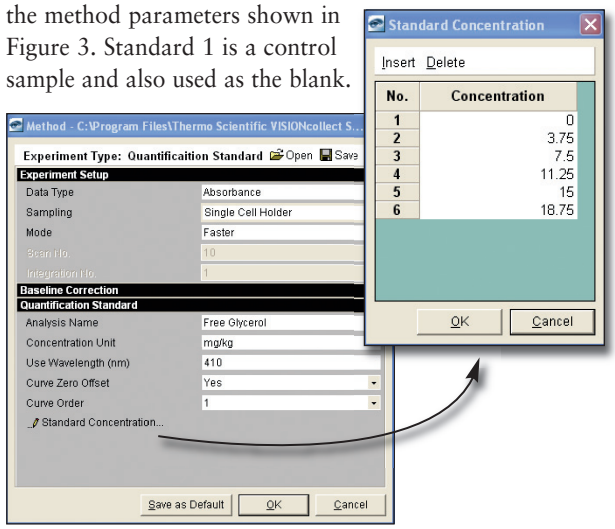


Figure 3: Method parameters for the measurement of free glycerol in biodiesel

The spectra and calibration curve obtained from the glycerol reference solutions are shown in Figure 4. A linear fit was applied, resulting in a correlation coefficient ($R^2$) value of 0.99817 and equation of y = 0.0524x – 0.0278. Using the calibration curve obtained, the free glycerol content in the sample was determined to be 2.11 mg/kg (Figure 5).

## Result and Conclusion

In this application note, we demonstrate the use of the Evolution™ Array™ UV-Visible spectrophotometer for the sensitive measurement of free glycerol in biodiesel fuels. In this case, the amount of free glycerol was determined to be 2.11 mg/kg or 0.000211%, which is well below the ASTM and EN limits of 0.02%.

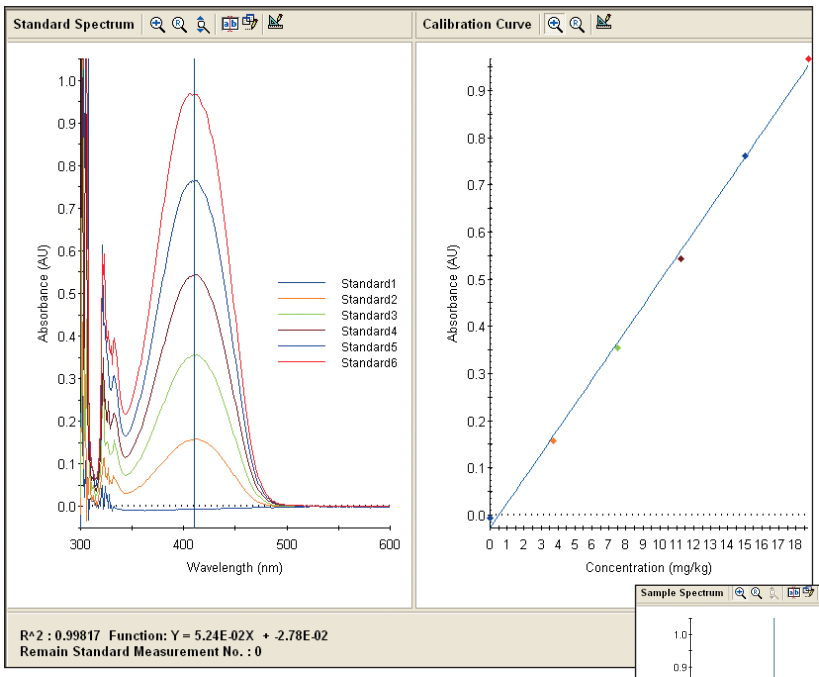


Figure 4: Standard spectra and calibration curve for glycerol

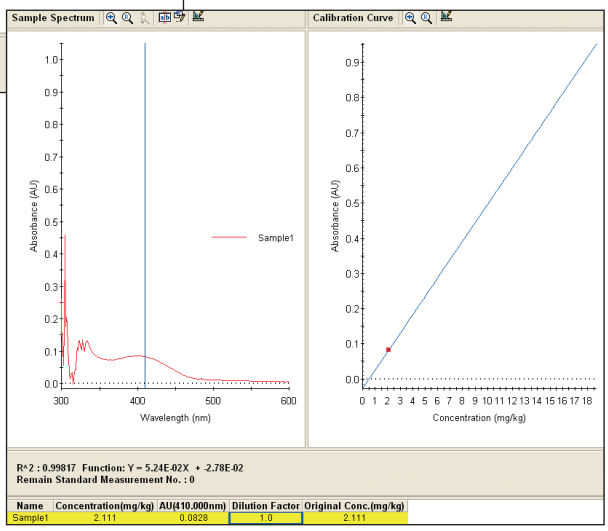


Figure 5: Sample spectrum and results

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4. Bondioli, P and Bella, L.D. (2005), *An Alternative Spectrophotometric Method for the Determination of Free Glycerol in Biodiesel*, Eur. J. Lipid. Sci. Technol. 107, 153-157.


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