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Bulletin 943A

Determination of Free and Total Glycerin and Moisture in B100 Biodiesel

Biodiesel is a renewable, alternative diesel fuel produced from vegetable oils, animal fats and recycled restaurant grease. This non-toxic, biodegradable liquid fuel consists of mono-alkyl esters of long chain fatty acids and may be used alone or blended with petroleum-based diesel fuels.

The most common method for producing biodiesel is the transesterification of fatty acid glycerol esters, commonly referred to as triglycerides. Fats and oils are made up of triglycerides. The triglycerides are chemically reacted with methanol using either sodium hydroxide or potassium hydroxide as a catalyst. The products of this reaction are mixed, long chain, mono-alkyl fatty acid methyl esters and glycerol (glycerin), which are then separated and purified. The resulting fatty acid methyl esters are biodiesel while the glycerol is often sold for use in soaps and other products. Biodiesel contains no sulfur or aromatic hydrocarbons and is almost 10% oxygen, making it an oxygenated fuel which aids combustion in fuel-rich circumstances.

Biodiesel that is 100% fatty acid methyl esters is called B100. The B100 biodiesel is recognized worldwide as an alternative fuel and qualifies for mandated programs in both the EU and US. Although there are many required tests, most producers use the concentration of free and total glycerin as a critical pass/fail test for B100. Standard methods ASTM D6584 or EN 14105 measure the free and total glycerin in B100 biodiesel. If the fuel does not pass, the biodiesel is either reworked or blended before further testing. Another important parameter in the production of B100 biodiesel is moisture content. Both of these tests are discussed in detail below.

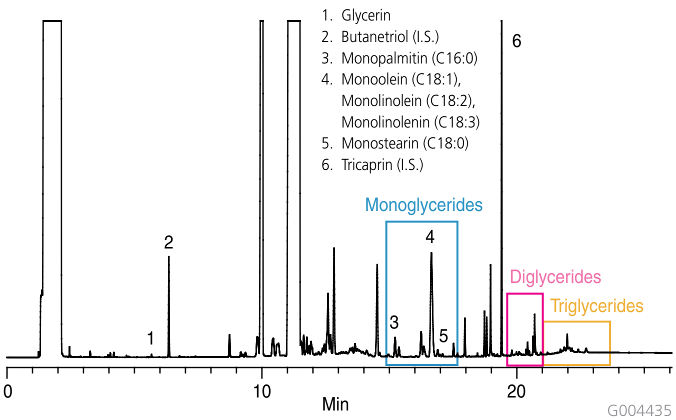
Free and Total Glycerin in B100 Biodiesel

Left in the fuel, glycerin can cause clogged fuel systems, injector deposits, filter plugging and build-up in the vehicle's fuel tanks. Therefore, low levels of free and total glycerin are critical to the specifications of biodiesel fuels. High levels of free and total glycerin are caused by the improper or low conversion of oil or fat into the desired mono-alkyl esters.

The biodiesel assay for free and total glycerin is outlined in methods ASTM D6584 and DIN EN 14105. These methods provide for the quantitative determination of free and total glycerin in 100% biodiesel fuel (B100 methyl esters) by high temperature gas chromatography after silylating the sample with N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA). An example of the GC analysis of biodiesel following ASTM D6584 methodology is shown in Figure 1.

Figure 1. B100 Biodiesel Sample on the MET-Biodiesel

column: MET-Biodiesel, 14 m x 0.53 mm I.D., 0.16 µm with integrated 2 m x 0.53 mm I.D. guard (28668-U)
oven: 50 °C (1 min.), 15 °C/min. to 180 °C, 7 °C/min. to 230 °C, 30 °C/min. to 380 °C (10 min.)
det.: FID, 380 °C
carrier gas: helium, 3.0 mL/min.
injection: 1 µL, cold on-column
sample: B100 Biodiesel plus Butanetriol Internal Standard (44896-U) and Tricaprin Internal Standard (44897-U), derivatized with MSTFA (394866) then diluted in heptane



Methods ASTM D6584 and DIN EN 14105 Calibration Standards

Determining the quality of newly processed B100 requires the use of two internal standards and multi-component calibration solutions, each containing glycerin, monoolein, diolein, and triolein, in varying concentrations. Analysts following method ASTM D6584 are required to prepare five different multi-component solutions, while those adhering to method DIN EN 14105 must prepare only four different multi-component solutions. Due to the possible overlapping of methyl ester and monoglycerides peaks in the chromatography, EN 14015 also recommends using a commercial monoglyceride mixture to aid in peak identification. This commercial monoglyceride mixture contains monopalmitin, monostearin and monolein. Although these chemicals are readily available, the preparation can be time consuming and requires working with pyridine.

Calibration standards preparation for both methods is made easier with pre-made Supelco brand multi-component varied concentration solutions. Use of these standards saves the analyst hours and reduces exposure to pyridine solvent. They still require care in following the included step-by-step procedures. These procedures are purposefully made similar to preparing B100 biodiesel samples to insure similar techniques to reduce error in preparation. Standards preparation includes pipeting a known amount of the calibration mix into a vial, adding both internal standards (butanetriol and tricaprin), and derivatization with MSTFA. After allowing the standards mixtures 20 minutes to fully derivatize, each standard is diluted with n-heptane. The standards are now ready for analysis by gas chromatography (GC).

Derivatization Reagents

Precautions for derivatization are important because derivatization-grade MSTFA is extremely sensitive to moisture. Purchasing and using the smallest reagent package practical for the work at hand is strongly recommended. Storing the reagent in a tightly sealed container, such as a refrigerated desiccator, will provide the best shelf life. Carefully drying all glassware and syringes that will come into contact with the MSTFA reagent during sample preparation minimizes the opportunity for the MSTFA to become inactive.

To store the derivatized standards, blanket the vial's headspace with dry nitrogen, tightly seal the vial, and place in a refrigerated area. Carefully stored standards have been shown to be stable for up to one-week. Refrigerated standards must be brought to room temperature prior to analysis.

MET-Biodiesel Capillary GC Column

The MET-Biodiesel was designed specifically for the determination of free and total glycerin in B100 biodiesel samples. Features and benefits of this column include:

- Provides good peak shape and resolution for all glyceride impurities of interest.
- Able to separate glycerin in addition to mono-, di-, and triglycerides (as methyl esters) from the FAMEs.
- A maximum temperature of 380 °C (isothermal) and 430 °C (programmed) exceeds the temperature limitations specified in biodiesel methods such as ASTM D6584 and EN 14105.
- Metal was selected as the column material because it holds up better than fused silica under the method conditions, virtually eliminating column breakage.
- The integrated guard will protect the analytical column from excess reagent and non-volatile compounds, extending column life with a leak-free connection.

Cool-on-column (COC) injection beginning at 50 °C is used with this method. Use of a heated injection port can lead to sample discrimination and is not suggested as a replacement for COC. The syringe needle used in this method must have a diameter small enough to fit inside the 0.53 mm I.D. guard column. Automated injection is highly recommended.

Materials with boiling points higher than triglyceride CN58 may not properly volatilize under the conditions of this method. These high boiling compounds, along with other non-volatile substances will deposit on the head of the guard column. Periodic trimming of the guard column will increase the life of the analytical column. Injection of samples containing too much non-volatile material will adversely affect the lifetime of the analytical column and is to be avoided. The source of many of these high boiling compounds is unreacted triglycerides due to incomplete transesterification or fatty acids that have not been completely esterified with MSTFA. Making sure the derivatization of standards is complete is critical for proper analysis and assuring column lifetime.

While proper sample preparation is highly important, minimizing contamination of carrier gas is equally critical. Leak-free connections prevent contamination of the carrier gas with air. Use of high purity carrier gas from a reputable supplier and in-line gas purifiers is a must in maintaining analytical column function and extending column lifetime.

Moisture Content in B100 Biodiesel

Water in biodiesel comes from several sources. Some of the water is residual from processing while some comes from condensation in the storage tanks. Accurate determination of water content is necessary for processing since the presence of water in biodiesel poses problems for a number of reasons.

- Water reduces the heat of combustion. This means more smoke, harder starting and less power.
- Water corrodes vital fuel system components, like fuel pumps, injector pumps, fuel lines, etc.
- Water forms ice crystal when temperature approaches 0 °C (32 °F). These crystals provide sites for nucleation and accelerate gelling of the residual fuel.
- Water is required for the growth of most microbes. The presence of water in biodiesel (an ideal nutrient base) accelerates the growth of microbe colonies that can clog fuel systems. Biodiesel users who have heated fuel tanks face a year-round microbial growth problem.

Although there are other methods, in practice, used for determination of water content in biodiesel, Karl Fischer titration using HYDRANAL reagents offers rapid, precise, reliable and reproducible water measurements. Using HYDRANAL reagents, water content in biodiesel can be determined by means of volumetric or coulometric titration, or by the Karl Fischer oven method.

Karl Fischer Titration Chemistry

The Karl Fischer technique for water determination is a titration reaction based on the Bunsen equation:

$$ROH + SO_2 + R'N = [R'NH]SO_3R \qquad (1)$$

$$[R'NH]SO_3R + H_2O + I_2 + 2R'N = 2[R'NH]I + [R'NH]SO_4R \qquad (2)$$

(R'N = base, ROH = alcohol, typically methanol)

Oxidation of alkylsulfite to alkylsulfate in reaction (2) consumes water, which should come only from the sample. Since water and iodine (I_2) are consumed in a 1:1 stoichiometric ratio, the amount of water in the original sample is calculated by measuring the amount of I_2 consumed until the endpoint is achieved. Iodine concentration is measured either volumetrically or coulometrically.

Biodiesel contains components consisting of various chain lengths. Therefore, a solubilizer is often required to dissolve or disperse it, in order to extract the water. Generally a medium containing chloroform (HYDRANAL LipoSolver CM) is considered a more effective solubilizer. Other products, such as, 1-hexanol based HYDRANAL LipoSolver MH, HYDRANAL Methanol Rapid or HYDRANAL–Solvent can also be used as a solubilizer depending upon the biodiesel component chain length.

Volumetric Titration

In general, HYDRANAL Composite 2 is used as a titration agent in the volumetric method. A 30 mL sample of a solubilizer media of choice is placed in the titration vessel. A 5 mL precisely measured sample of the biodiesel under testing is injected into the media. The mix is then titrated with HYDRANAL Composite 2 to determine the water content.

Biodiesel components contain double bonds that can react with iodine and lead to erroneously high results. Generally, there is only a small tendency towards this side reaction and is not significant enough to interfere with the analysis. Falsely high results are evident by extremely fading end points, a typical sign of the side reaction.

Coulometric Titration

The coulometric procedure is more sensitive. Coulometric determination of water content is carried out in a cell with or without a diaphragm. A volume of 5 mL of biodiesel, precisely weighed using the differential weighing method, is recommended as a test sample. The precision of the sample manipulation and the coulometric cell can be tested by means of HYDRANAL Water Standard 0.10.

Procedure

Using cell with diaphragm: HYDRANAL Coulomat CG (5 mL) is placed in the cathode chamber of a coulometric cell and approximately 100 mL HYDRANAL Coulomat Oil is placed in the anode chamber up to the same level. The coulometer is switched on, and the cell is automatically titrated to dryness. When the drift is stabilized at <10 µg/min., a 5 mL sample, precisely measured using differential weighing, is injected into the titration vessel and titrated to obtain the water content.

Using cell without diaphragm: The titration follows the same procedure as for coulometry in a cell with diaphragm except that in this titration, HYDRANAL Coulomat AG-H is used as the only reagent instead of HYDRANAL Coulomat CG and HYDRANAL Coulomat Oil.

Water Content Using the Karl Fischer Oven

Our tests indicated that 100 °C is a suitable temperature for releasing water from biodiesel in a Karl Fischer oven. At about 120 °C, a slight side reaction occurs and at 190 °C the sample emits smoke and decomposes.

To prepare the coulometer for use with the Karl Fischer Oven, add 100 mL of HYDRANAL Coulomat AG Oven to the anode chamber. For instruments equipped with a diaphragm generator electrode, add 5 mL of HYDRANAL Coulomat CG to the cathode chamber. Switch the instrument on and allow to condition until the drift stabilizes at less than 10 µg/min. A 4 mL precisely weighed biodiesel sample is evaporated at 100 °C.

HYDRANAL Molecular Sieve 0.3 nm is well suited as a drying medium for the carrier gas.

HYDRANAL Contact Information

For technical help on HYDRANAL in USA and Canada, please contact our Karl Fischer help line:

Mr. Doug Clark
HYDRANAL Technical Center
545 S. Ewing Ave.
St. Louis, MO 63103
Tel.: 800-HYDRANAL (toll-free hotline)
Fax: 314-286-6699
e-mail: doug.clark@sial.com

Ordering Information:

Description	Cat. No.
Internal Standards	
Butanetriol (CAS# 42890-76-6), 1000 µg/mL in pyridine, 5 mL	**44896-U**
Tricaprin (CAS# 621-71-6), 8000 µg/mL in pyridine, 5 mL	**44897-U**
ASTM D6584 Individual Calibration Standards and Kits	
Glycerin (CAS# 56-81-5), 5000 µg/mL in pyridine, 1 mL	**44892-U**
Monoolein (CAS# 111-03-5), 5000 µg/mL in pyridine, 3 mL	**44893-U**
Diolein (CAS# 2465-32-9), 5000 µg/mL in pyridine, 2 mL	**44894-U**
Triolein (CAS# 122-32-7), 5000 µg/mL in pyridine, 2 mL	**44895-U**
ASTM D6584 Individual Standard Kit with Internal Standards	**44898-U**
Kit contains 1 ea: 44892-U, 44893-U, 44894-U, 44895-U, 44896-U, and 44897-U	
ASTM D6584 Calibration Standard Mixes and Kits	
ASTM D6584 Standard Solution 1, in pyridine, 1 mL Diolein, 50 µg/mL Glycerin, 5 µg/mL Monoolein, 100 µg/mL Triolein, 50 µg/mL	**44899-U**
ASTM D6584 Standard Solution 2, in pyridine, 1 mL Diolein, 100 µg/mL Glycerin, 15 µg/mL Monoolein, 250 µg/mL Triolein, 100 µg/mL	**44914-U**
ASTM D6584 Standard Solution 3, in pyridine, 1 mL Diolein, 200 µg/mL Glycerin, 25 µg/mL Monoolein, 500 µg/mL Triolein, 200 µg/mL	**44915-U**
ASTM D6584 Standard Solution 4, in pyridine, 1 mL Diolein, 350 µg/mL Glycerin, 35 µg/mL Monoolein, 750 µg/mL Triolein, 350 µg/mL	**44916-U**

Description	Cat. No.
ASTM D6584 Standard Solution 5, in pyridine, 1 mL	**44917-U**
Diolein, 500 µg/mL	
Glycerin, 50 µg/mL	
Monoolein, 1000 µg/mL	
Triolein, 500 µg/mL	
ASTM D6584 Standard Solution Kit with Internal Standards	**44918-U**
Kit contains 1 ea: 44899-U, 44914-U, 44915-U, 44916-U, 44917-U, 44896-U, and 44897-U	
EN 14105:2003 Calibration Standard Mixes and Kits	
EN 14105:2003 Monoglyceride Stock Solution, in pyridine, 1 mL	**49446-U**
Monoolein, 10 mg/mL	
Monopalmitin, 10 mg/mL	
Monostearin, 10 mg/mL	
EN 14105:2003 Standard Solution 1, in pyridine, 1 mL	**49441-U**
Butanetriol, 80 µg/mL	
1,3-Diolein, 50 µg/mL	
Glycerol, 5 µg/mL	
Monoolein, 250 µg/mL	
Tricaprin, 800 µg/mL	
Triolein, 50 µg/mL	
EN 14105:2003 Standard Solution 2, in pyridine, 1 mL	**49442-U**
Butanetriol, 80 µg/mL	
1,3-Diolein, 200 µg/mL	
Glycerol, 20 µg/mL	
Monoolein, 600 µg/mL	
Tricaprin, 800 µg/mL	
Triolein, 150 µg/mL	
EN 14105:2003 Standard Solution 3, in pyridine, 1 mL	**49443-U**
Butanetriol, 80 µg/mL	
1,3-Diolein, 350 µg/mL	
Glycerol, 35 µg/mL	
Monoolein, 950 µg/mL	
Tricaprin, 800 µg/mL	
Triolein, 300 µg/mL	
EN 14105:2003 Standard Solution 4, in pyridine, 1 mL	**49444-U**
Butanetriol, 80 µg/mL	
1,3-Diolein, 500 µg/mL	
Glycerol, 50 µg/mL	
Monoolein, 1250 µg/mL	
Tricaprin, 800 µg/mL	
Triolein, 400 µg/mL	
EN 14105:2003 Standard Solution Kit	**49445-U**
Kit contains 1 ea: 49441-U, 49442-U, 49443-U, and 49444-U	

Trademarks

HYDRANAL, Supelco — Sigma-Aldrich Biotechnology LP

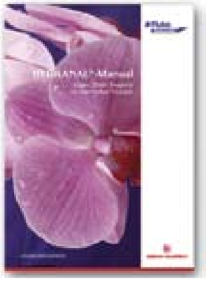
Description	Cat. No.
MET-Biodiesel Capillary GC Column	
14 m x 0.53 mm I.D., 0.16 µm with integrated 2 m x 0.53 mm I.D. guard	**28668-U**
Derivatization Reagents and Solvents	
MSTFA, derivatization grade, 5 mL	**394866-5ML**
MSTFA, derivatization grade, 10 x 1 mL	**394866-10X1ML**
MSTFA, derivatization grade, 25 mL	**394866-25ML**
n-Heptane, ReagentPlus, 99%, 1 L	**H2198-1L**
n-Heptane, ReagentPlus, 99%, 4 L	**H2198-4L**
n-Heptane, ReagentPlus, 99%, 4 x 4 L	**H2198-4X4L**

HYDRANAL®	
HYDRANAL Composite 2	**34806**
HYDRANAL LipoSolver CM	**37855**
HYDRANAL LipoSolver MH	**37856**
HYDRANAL Methanol Rapid	**37817**
HYDRANAL Coulomat A	**34807**
HYDRANAL Coulomat Oil	**34868**
HYDRANAL Coulomat AG-H	**34843**
HYDRANAL Coulomat AG Oven	**34739**
HYDRANAL Coulomat CG	**34840**
HYDRANAL Solvent	**34800**
HYDRANAL Water Standard 0.10	**34847**
HYDRANAL Molecular Sieve 0.3 nm	**34241**

Literature

HYDRANAL Manual (IPC)

A 128-page manual containing theory and practical advise on Karl Fischer Titration.



HYDRANAL – Multimedia Guide CD (GVJ)

This guide covers nine different subjects and features a video-assisted description of volumetric and coulometric titration and a complete product listing. Over 500 applications and 400 laboratory reports on specific samples, detailed instructions designed according to ISO 9000.

Additional information can be found at our website: sigma-aldrich.com/biofuels

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