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ORIGINAL PAPER

Determination of Free Glycerol in Biodiesel via Solid-Phase Extraction and Spectrophotometric Analysis

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Abstract To date, the standardized method for glycerol quantification in biodiesel production utilizes gas chromatography (GC); however, availability to manufacturers and instrumentation cost limits GC as an analytical method for general quality enforcement among producers. The method developed here is a bench top technique for quantitative determination of glycerol in biodiesel, with practical application in pharmaceutical and environmental quality control. The method extracts the glycerol contaminant from biodiesel using a normal phase solid phase extraction column (SPE). The protocol proceeds by rinsing with hexane to remove residual methyl esters, then collecting the glycerol with water. The aqueous extract is analyzed spectrophotometrically by an anthrone coloring reagent. Use of 2-g SPE columns and the solvent system developed has achieved 85% glycerol recovery. The assay applied has a detection range of 0.004–0.400% free glycerol, comparable to the established American Society of Testing and Materials (ASTM) D 6584-07 GC technique. Results were confirmed by GC and high-pressure liquid chromatography (HPLC). The bench top technique reduces the costs of operation relative to current methods, completes analysis in proficient time, requires minimal labor, and has analytical limits comparable to existing standard methods of biofuel analysis.

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Introduction

Growing concerns over dependency on foreign fuel and the depleting health of the environment have urged US researchers, government bodies, and individual consumers alike to give more priority to finding alternative energy sources. Biodiesel is an alternative fuel that already has been introduced for mainstream consumption [1, 2]. As defined by the National Renewable Energy Laboratory (NREL), biodiesel is a fuel comprised of mono-alkyl esters of long-chain fatty acids derived from the transesterification of triglycerides, vegetable oils and/or animal fats, with short chain alcohols, as depicted by Scheme 1 [1].

Basic biodiesel processing has been understood since the early 1940s though only marginally utilized or further developed until recent decades due to cheaper costs and availability of petroleum-based fuels. Nonetheless, derivatizing oils and fats results in a fuel with properties similar to petroleum-based diesel and a product that can be distributed as an alternative to conventional fuel [3–5]. Due to its easy adaptability into mainstream operation, biodiesel is a very promising alternative fuel prospect for possible increased production in the future, both by large scale production facilities and individual consumers.

Specific methods for synthesizing biodiesel vary among producers. Among the most commonly utilized methods include homogeneous base catalysis with sodium hydroxide or sodium methoxide; homogeneous acid catalysis by sulfuric acid, phosphoric acid, hydrochloric acid, or organo-sulfonic acids; and more recently, ongoing

development of heterogeneous acid and base catalysis including metal complexes of Sn^{2+}, Zn^{2+}, and Pb^{2+}, and aluminium and titanium silicates [3, 6–8]. Each synthesis method results in a range of alcohol ester conversions and various by-products, necessitating different post-treatments and washing steps. Due to the assorted processing methods, the reactants and various grades of feedstock, the size of the different operations, and the range of distribution, establishing national and international standards, and further maintaining them, is a challenge [9, 10]. Nonetheless, quantitative analysis of the final product is essential for determining the quality of the biodiesel and permitting transactions between manufacturers, distributors, and consumers.

The American Society of Testing and Materials (ASTM) has defined the standards for biodiesel in method ASTM D 6751-09, though strict universal enforcement of the specifications is lacking. For industrial processing plants providing for the general market, meeting the standards is the responsibility of the producer to the consumer. However, the costs of running all the quality tests is extensive and often deters large producers from performing complete ASTM analysis. There is a great incentive to provide affordable analytical techniques for producers that are easy to operate and ensure overall, accurate product quality.

In particular, focus on the quality determination of biodiesel as a whole, and specific to this study, is the free glycerol count measured by ASTM D 6584-09. The ASTM limit allows a maximum of 0.020 wt% free glycerol in the biodiesel product. Quantification of glycerol is an indicative of how far the reaction has gone to completion and is valuable to the producer in an optimization of the reaction. Free glycerine results from incomplete separation of the final ester product and glycerol by-product after the transesterification reaction and water washing or other methods used for separation. The by-product creates problems in storage and can be a source of carbon deposit in engines due to incomplete combustion. The availability of an accurate and efficient technique for glycerol analysis would greatly enhance overall production.

ASTM D 6584-09 outlines the technique for the determination of free glycerine in B-100, 100% biodiesel fuel, and biodiesel/petro-diesel blends such as B-2 or B-10, where 2 and 10% are biodiesel, respectively. While, GC analysis requires only a small quantity of the sample and has low detection limits, the accuracy and reproducibility of the method cause uncertainty among producers. The samples require silylating for detection and multiple internal standards are necessary, adding to time and monetary investment. Further, due to instrumentation cost most biodiesel producers do not have GC facilities available on site. This requires manufacturers to send out samples for analysis prior to distribution which impedes sale interactions, and for smaller, individual producers often no analysis is carried out at all. Yet to date, this is the only standardized method for glycerol quantification in biodiesel samples. Development of an analytical method which requires minimal instrumentation cost and operation, and one that can be performed within a reasonable timeframe onsite would allow for better enforcement of standard requirements among all producers and greater quality assurance for consumers.

Experimental Procedure

Extraction Materials and Conditions

Stock glycerol (Fischer Scientific, Fairlawn), hexane (HPLC grade, Fischer Scientific), methanol (reagent grade, Fischer Scientific), deionized water, 99.0% sulfuric acid (analytical grade, Fischer Scientific), and anthrone (Fischer Scientific) were used without further purification. One and two gram normal phase silica solid phase extraction (SPE) (Alltech, Fisher, Deerfield) columns with a 1 wt% loading capacity were used for method development. Conditioning of columns was by rinsing with hexane according to the manufacturer, and development of the mobile phase is described. Preparation of the anthrone assay was according to Pons et al. and was used in developing a standard concentration curve for spectrophotometric detection and application for analysis of the extracted samples [12].

For separation, processed biodiesel samples of 2.5 g were mixed with 2 mL methanol. Samples were loaded onto conditioned columns and allowed to gravity load and filter. Columns were rinsed with 20 mL hexane, again by gravity filtration, to remove the methyl esters and other nonpolar residual material. Vacuum was applied to dry the column of the nonpolar solvent and prepare it for a polar solvent. Glycerol was collected from column experimentally by a combination of polar solvents to determine the most efficient means of removing the analyte and preparing it for analysis. Solvents included: deionized water, 0.5 N HCl, 1.0 N HCl, methanol, acetone, and 10% H_2SO_4, with amounts ranging from 5 to 15 mL, and with and without applied vacuum.

Vacuum filtration was applied at 17 kPa to elute the collecting solvent through the column. The collected eluent was concentrated by removing the solvent under air. Samples were reconstituted with 0.5 mL deionized water for spectrophotometric analysis against a standard glycerol curve.

Spectrophotometric Analysis

Stock glycerol amounts of 0.10, 0.50, 2.5, 5.0, 7.5, and 12.16 ppm were used in developing a standard curve. Five

hundred microliters of each concentration in water was developed with 5 mL of prepared anthrone assay. Mixtures were heated in a hot water bath for 10 min to develop color; cooled in ice for 10 min; and then set at room temperature for 10 min before spectrophotometric analysis. Quantitative analysis was measured at 510 nm against a water blank developed by the SPE columns under the same conditions. A standard curve, concentration versus absorbance, was generated and the stock glycerol control and experimental samples compared against it.

The spectrophotometric method developed was confirmed by high-performance liquid chromatography (HPLC) with a refractive index detector (RI) and gas chromatography mass spectroscopy (GC–MS) analysis. For chromatographic analyses, glycerol was extracted from biodiesel samples with SPE columns by the final developed protocol to confirm that the glycerol analyte was being selectively collected in the separation process.

HPLC Analysis

Stock glycerol and biodiesel samples were developed using SPE columns in the same way and concentrated under air. Samples were reconstituted in 1.0 mL of water for analysis by HPLC equipped with RI detector. An Aminex® HPX-87H, 300 mm × 7.8 mm column purchased from Bio Rad was used with an isocratic mobile phase of 0.01 N H_2SO_4 at 0.6 mL/min. The column temperature was held at 60.0 °C with the reactor temperature maintained at 50.0 °C. Runtime was set at 18 min. A standard curve of concentration versus area of peak was developed to determine the concentration of the glycerol removed from the SPE columns. The percentage recovered determined by HPLC analysis was compared to the results from the spectrophotometric method.

GC Analysis

Glycerol samples were developed according to the final spectrophotometric method and prepared for analysis according to the ASTM: D 6584-09 protocol. Silylation of standard glycerol samples and collected samples with *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) and dilution with heptane was done as outlined in the ASTM method. An open tubular column with 5% phenyl polydimethyl trifluoroacetamide siloxane was used with a helium carrier gas. Column temperature was set at an increasing gradient from 50 to 180 °C, with a 15 °C/min increase. Detector temperature was maintained at 180 °C. The run time was 13 min. Derivatized trimethylsilyl ether of glycerol was obtained for GC–MS analysis and the area of the peak was used in quantification. GC analysis was used for comparison to the developed spectrophotometric method, in conjunction with HPLC analysis.

Results and Discussion

The bench top spectrophotometric method developed provides an alternative means of biodiesel analysis that is both affordable and operable among the broad range of biodiesel producers. The method uses normal phase SPE columns to separate the glycerol by-product and the biodiesel product. An anthrone assay is applied for quantitative spectrophotometric analysis. The method compares favorably against published HPLC methods and the ASTM GC method.

Development of the delivery technique and mobile phase was as follows: to load the column, the standards and biodiesel samples were dissolved in methanol and allowed to gravity filter through a conditioned column. The SPE column must first be eluted with a solvent to remove the nonpolar compounds, followed by a reagent for collecting the polar glycerol analyte for analysis. Hexane was applied initially for rinsing the column of the nonpolar methyl esters and 20 mL proved to be the minimum amount necessary for removing nonpolar substances. Acetone, 1.0 N and 0.5 N HCl, and water were all experimented with individually for collection of glycerol from the column. Acetone showed itself to interfere with the anthrone reagent in the analysis and concentrations of hydrochloric acid were destructive toward the column in the process of removing the glycerol. Though deionized water requires more time for concentration of the samples under air, recovery averaged 85%. Water blanks caused no interference with the assay. Thus, water was used for the elution of glycerol from the column. To justify use of the 10 mL of water, 5 mL of water and 15 mL of water were also used in eluting the glycerol from the column. It was found that the percentage recovery increased by an average of 6.7% from 5 to 10 mL extractions with no significant difference increasing to 15 mL. To maintain a reasonable time of analysis, 10 mL has become the protocol for glycerol recovery. In development of the method, both 1- and 2-g

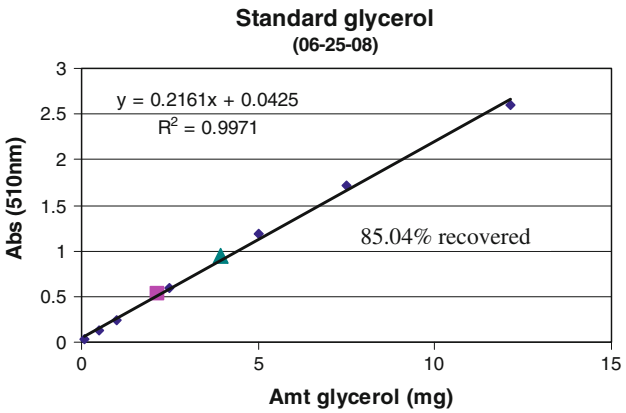

Fig. 1 Spectrophotometric analysis of glycerol extracted from biodiesel by normal phase solid phase extraction (SPE) column

Table 1 Comparison of spectrophotometric, HPLC, and GC analysis of glycerol extracted from biodiesel by solid phase extraction SPE

Method	Average stock glycerol recovered (%)	Average % by weight BD glycerol recovered	Average % by weight BD glycerol, with recovery factor
Spectrophotometric detection	85.04 ± 0.23	0.15 ± 0.05	0.17
HPLC	81.65 ± 1.9	0.16 ± 0.01	0.19
GC–MS	84.00 ± 0.76	0.19 ± 0.06	0.22

normal phase SPE columns were applied, with analyte capacities of 1% column weight for each. Figure 1 shows the graph of spectrophotometric recovery results, where (filled square) represents the amount recovered from the 1-g column and (filled triangle) the amount recovered from the 2-g column. The same solvent scheme was used in developing each of the columns: washing with hexane and collecting with 10 mL water for analysis. On an average 2-g columns recovered 40% more glycerol analyte and thus were determined the columns for the final method. Trials that allowed gravity filtration provided highest recovery results.

The assay chosen for glycerol analysis of biodiesel had been used previously in glycerol quantification in biological samples. Anthrone is a quinone glucoside capable of hydrogen bonding with oxygen atoms within carbon–oxygen molecules in an acidic environment. The conjugation creates a field that is detectable spectrophotometrically between 475 and 510 nm [13]. The anthrone assay applied for glycerol analysis can be used in detection of ethylene glycol and other contaminants of pharmaceuticals and environmental samples with revision to the method presented. Preliminary tests have determined that ethylene glycol is detectable at the same lower limit as glycerol. For application purposes in the lab, detection limits of the assay must be comparable to the standardized GC–FID method.

A standard curve was generated at the appropriate time and wavelength measured for quantification of the eluted samples. The standard curve generated in Fig. 1 has a favorable R^2 value for eluted samples to be compared against. From the 2-g column, 85% recovery was observed. Amounts of glycerol are expected to be lost in loading the columns, as sample may not be recovered from the walls and not be rinsed through the normal phase for extraction. Also in drying the sample under air, glycerol may be not be concentrated down from the walls of the vials; and further, 10 mL of water may not be sufficient to remove 100% glycerol under these conditions, though the amount is most reasonable considering the drying time.

The spectrophotometric method developed for free glycerine analysis results in a range of detection of 0.004–0.400 mass% free glycerine, which is comparable to the standard GC method of 0.005–0.500 mass%. It can be performed at reasonable cost with basic spectrophotometric instrumentation and can be completed in minimal time. Detection of the biodiesel glycerol by-product by spectrophotometric means was achieved at levels comparable to the established ASTM GC technique and after comparative analysis against GC and HPLC, it has been determined that the analytical method developed here can be applied with confidence.

GC–MS and HPLC analyses were used to confirm the developed method of glycerol quantification. Samples of stock glycerol and biodiesel were developed under the same SPE column protocol, concentrated, and analyzed by an HPLC published by the NREL and according to GC conditions outlined by ASTM [10, 11]. The standard curve in HPLC analysis was adjusted to meet the amounts of analyte injected onto the column. The range of detection is 0.0002–0.04 mass% glycerol. Similarly, recovery of glycerol from the column was seen and the comparison of the two methods is presented in Table 1. As GC and HPLC are more sensitive analytical tools, it was expected to see a lower recovery as a result of the same factors presented above.

Conclusion

For the general biodiesel market, standards must be upheld, thus analytical techniques must be made feasible for the producers if the aim is to continue positively impacting the fuel situation. Availability of a bench-top method for glycerol analysis of biodiesel benefits the producers and the consumers. It can be performed onsite for biodiesel manufacturing with basic lab instrumentation and minimal labor intensity. For large scale producers, the method would allow analysis to be completed in a timely manner, thus increasing production efficiency and ultimately sales. Though the method is specific to biodiesel analysis, it can be adapted for analysis of pharmaceuticals and environmental contaminants. Concentration curves specific to the analyte of interest can be developed accordingly and quantitative amounts of samples determined thus benefitting many industries.

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