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Analysis of Biodiesel Quality Using Reversed Phase High-Performance Liquid Chromatography

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ANALYSIS OF BIODIESEL QUALITY USING REVERSED PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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Abstract

The alternative fuel biodiesel is produced from the transesterification of vegetable oils or animal fat to fatty acid methyl esters. Pomona has a reactor on campus that can be used to run this reaction and produce biodiesel. The use of biodiesel has been found to lower air pollutant and greenhouse gas emissions, but can be potentially harmful to the engines if it contains impurities. This paper proposes a method using high-performance liquid chromatography to test the quality of biodiesel. This method utilizes instrumentation and materials that are available in Pomona College's Chemistry Department, requires very little sample preparation, and is relatively safe, as long as general lab safety practices are followed. This method can also be used to optimize the procedure used to make the biodiesel. An optimized production procedure and a test method to assess the final product will ensure high quality fuel that can be used with confidence in diesel engines. This will likely add strength to proposals to increase the use of the on-campus reactor and produce biodiesel for campus grounds equipment from waste vegetable oil.

Chapter 1 - Introduction

What is Biodiesel?

The alternative fuel known as biodiesel is defined as "fuel comprised of mono-alkyl esters of long chain fatty acids, derived from vegetable oils or animal fats, designated B100"[1]. Biodiesel is produced through a transesterification reaction. Vegetable oil or animal fat is reacted with an alcohol, usually methanol or ethanol, in the presence of a catalyst to yield fatty acid methyl esters (FAME), also known as mono-alkyl esters, and glycerol; the latter is later removed[1] (Figure 1).

| Triglyceride | Methanol | Glycerol | Fatty-Acid Methyl Ester |
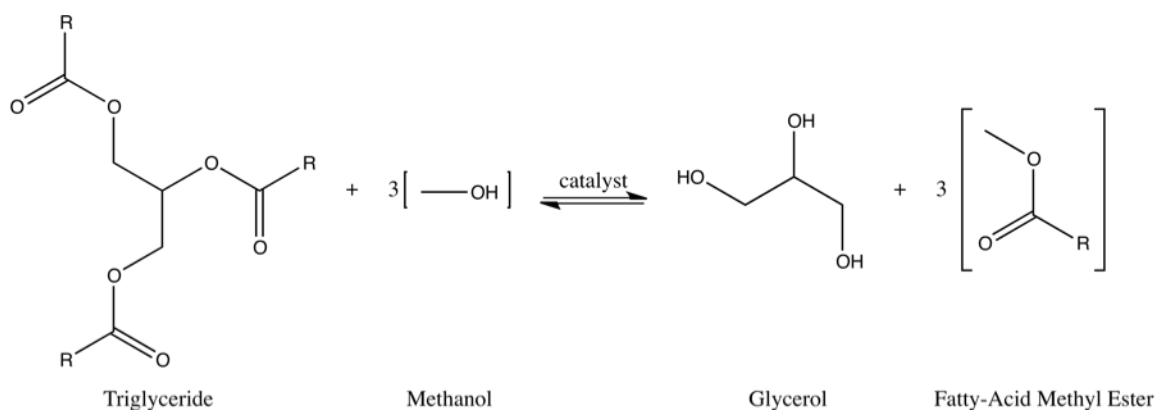
Figure 1. Transesterification reaction between a triglyceride and methanol, resulting in glycerol and fatty-acid methyl esters (FAME). The source of the triglyceride may be either vegetable oil or animal fat. Monoglycerides or diglycerides may also undergo this same reaction, using just 1 or 2 molar equivalents of methanol and producing 1 or 2 molar equivalents of FAME, respectively.

Petroleum-derived diesel is produced from the fractional distillation of crude oil. Distillation is a technique used to separate different chemical compounds based on differences in their vapor pressures. Fractional distillation utilizes a special fractionating column, which allows for the separation of multiple chemical compounds. Oil refineries use this process to separate out the many components of crude oil, which are used to make a variety of products. Diesel is a direct product obtained from the fractional

distillation of crude oil. It is the fuel oil that distills between 180 °C and 380 °C[2]. Due to growing demand for fuel, other processes, such as cracking, have also been adopted by refineries to increase the fractional portion of this fuel (Figure 2).

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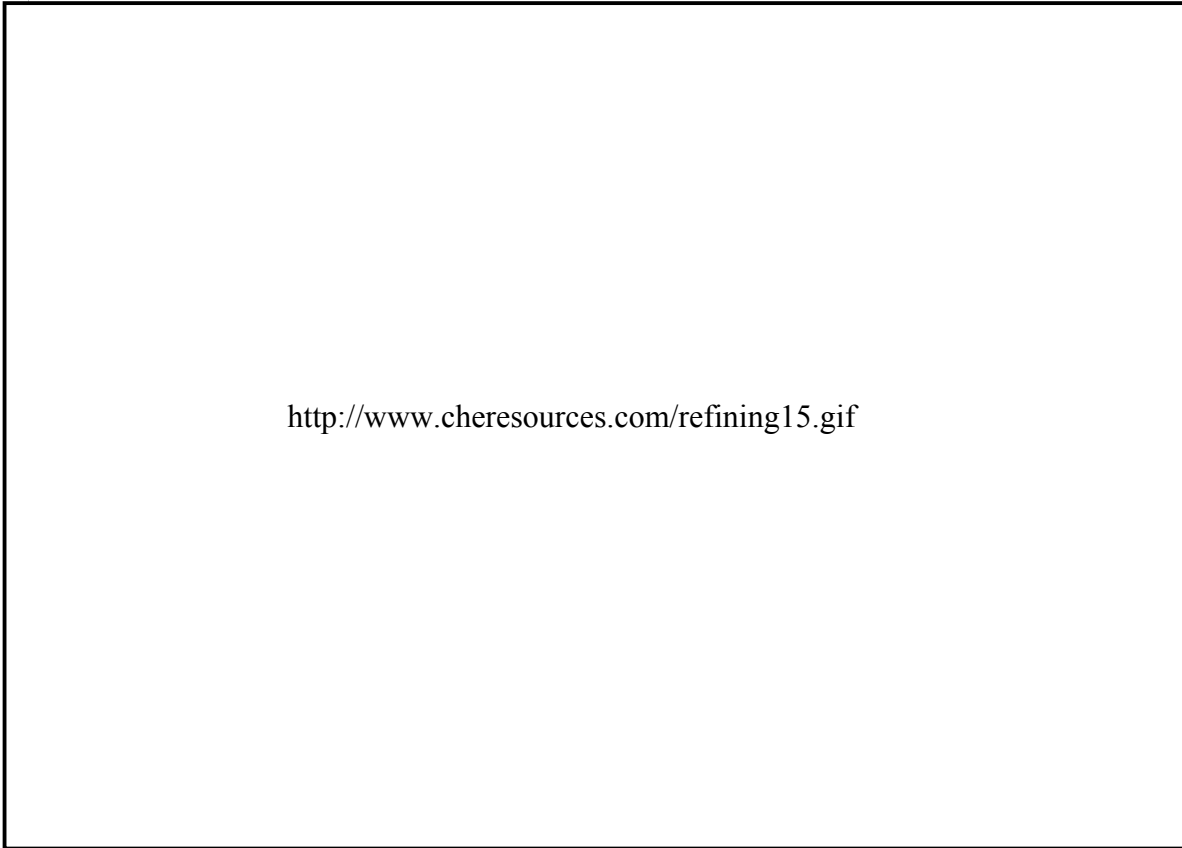
Figure 2. Schematic overview of an oil refinery. Products used as petrodiesel are outlined in red. Original image from OSHA Technical Manual, Section IV, Chapter 2, Petroleum Refining Processes.

Compared to diesel, biodiesel has a higher oxygen content, lower sulfur and aromatic content, and a higher cetane number, but all other crucial properties remain the same[3]. Biodiesel can be burned in diesel engines alone, or blended with traditional petroleum-derived diesel. The starting material for producing biodiesel can be a variety of different vegetable oils or animal fats, including used oil.

Environmental Benefits

As with any other fossil fuel, the use of diesel results in the emission of air pollutants and greenhouse gases. The use of biodiesel has been found to lower emissions of many of these air pollutants. Trucks running on a 35% biodiesel 65% diesel blend were shown to emit smaller amounts of particulate matter (PM), carbon monoxide (CO), and hydrocarbons (HC), than the same trucks fueled by no. 2 diesel. These emission reductions were obtained without reductions in engine performance or fuel economy[3]. Other studies have shown additional reductions in volatile organic compounds (VOC) and sulfur oxides (SO_x)[4]. These pollutants all have serious human health effects, either directly or through the production of ozone (Table 1). Replacing petroleum-derived diesel with biodiesel also reduces greenhouse gas emissions by 41%[4]. Currently, fossil fuels, including diesel, account for 56.6% of all greenhouse gas emissions in the United States in the form of carbon dioxide (CO_2)[5]. In addition to these immediate reductions in emissions, the production of biodiesel offers a method to recycle used vegetable oil, which would otherwise be thrown away.

Need for Test Method

Pomona has a reactor on campus, created by a group of students, which is capable of producing biodiesel from waste vegetable oil used in the dining halls and other campus eating establishments, such as the Coop Fountain and Sagehen Cafe. This offers an opportunity for the school to reduce the amount of waste it produces and to create a more environmentally friendly fuel to use in their diesel engines. The use of this fuel, however, can be potentially harmful to the engine if it contains impurities. Unreacted tri-, di-, and mono-acylglycerols, as well as residual free glycerol can cause fuel filters to clog and

Table 1. Common Air Pollutants from Diesel Fuel and Their Health Effects

Pollutant	Health Effects
Particulate Matter (PM)	• Increased respiratory symptoms (e.g. irritation of the airways, coughing, or difficulty breathing) • Decreased lung function • Aggravated asthma • Chronic bronchitis • Irregular heartbeat • Nonfatal heart attacks • Premature death in people with heart or lung disease[6]
Carbon Monoxide (CO)	• Reduced oxygen delivery to body organs and tissues • Myocardial ischemia (reduced O_2 to heart) • Death • Production of ozone[7]
Sulfur Dioxide (SO_2)	• Adverse respiratory effects (e.g. bronchoconstriction, increased asthma symptoms) • Production of PM[8]
Volatile Organic Compounds (VOC)	• Production of ozone[9]
Ozone	• Airway irritation, coughing, and pain when taking a deep breath • Wheezing and breathing difficulties during exercise or outdoor activities • Inflammation, which is much like a sunburn on the skin • Aggravation of asthma and increased susceptibility to respiratory illnesses like pneumonia and bronchitis • Permanent lung damage with repeated exposures[9]

may leave deposits in the engine, ultimately resulting in poor engine performance[10]. The

American Society for Testing and Materials (ASTM) has developed a standard indicating

the maximum amount of free and total glycerin that can be present in biodiesel without

adverse effects to the engine. The amount of free glycerol must be less than 0.02% (w/w)

and the amount of bound glycerol must be less than 0.24% (w/w)[1]. ASTM has also

published a standard test method to determine the free and total glycerin in biodiesel using gas chromatography (GC). This method, however, is not ideal for characterizing non-volatile triglycerides that may be present in the fuel. In order to record these compounds, the sample must be derivatized with N-methyl-N-trimethylsilyltrifluoro-acetimide (MSTFA), a very flammable and potentially dangerous chemical. This method also requires very high analysis temperatures (up to 380 °C)[11]. In order to perform analysis at these temperatures, a specialized high temperature column is needed, which can be very expensive. The purpose of this study is to develop a method to test biodiesel using the instrumentation and materials currently available on campus.

High Performance Liquid Chromatography

High performance liquid chromatography (HPLC) is another common technique used to analyze biodiesel. It has been used to identify various components of biodiesel mixtures including fatty acid methyl esters, triglycerides, diglycerides, monoglycerides, and fatty acids, among others. HPLC analysis time is generally shorter than GC, and no derivitization step is needed. Additionally, lower analysis temperatures allow for the use of standard columns[12]. In an HPLC analysis, the sample is injected into a column with a non-polar stationary phase. A polar mobile phase is then pumped through the column to elute the sample. As the sample moves through the column, the different components begin to separate based on polarity. The most polar compounds do not have a tendency to stick to the non-polar stationary phase, but instead move through with the polar mobile phase and elute quickly. Non-polar components stick to the stationary phase and are retained longer. A detector at the end of the column detects each compound as it is being

eluted. Retention times for each compound can be recorded and compared to retention times of known standards to identify the different components of the sample.

A variety of detectors can be used with this technique including UV detectors, refractive index detectors, evaporative light scattering detectors, or mass spectrometers. The Pomona College Chemistry Department has an HPLC equipped with both a UV detector and a mass spectrometer. By using two detection techniques, the advantages of each can be capitalized on. Ultra-violet (UV) detection relies on the presence of a chromophore, a functional group that absorbs UV light. Double bonds are chromophores and allow for the visualization of many of the compounds present in biodiesel. Fully saturated compounds, however, have no double bonds and thus do not absorb in the UV region, making them invisible to UV detection. Mass spectrometry, however, does not require the presence of a double bond for visualization. Often called a universal detector, the MS can analyze a wide range of compounds by ionizing the sample as it elutes and then separating the ions based on their mass-to-charge (m/z) ratio. This results in a mass spectrum for each eluted peak. This spectrum gives information about the size of each compound, helping to identify each peak on the chromatogram. The area of both UV and MS peaks are proportional to the concentration of each component in the sample, allowing for the quantification of each component in the biodiesel[13].

Chapter 2 - Experimental

Reagents and Materials

Mobile phase solvents included acetone Chromasolv for HPLC ≥99.9%, acetonitrile HPLC grade ≥99.93+%, and water dispensed from a Millipore Q-Pod system. 0.1% trifluoroacetic acid (TFA) ReagentPlus 99% was added to the mobile phases in order to increase ion formation for MS detection. All standards and samples were dissolved in either acetone Chromasolv for HPLC ≥99.9% or tetrahydrofuran (THF) anhydrous ≥99.9% inhibitor free. Acetone, acetonitrile, TFA, and THF were obtained from Sigma-Aldrich. Standards were obtained from Nu-Chek-Prep, Inc. (Elysian, MN, USA). These included fatty acids (linoleic acid, stearic acid, palmitic acid, oleic acid), methyl esters (methyl linoleate, methyl oleate, methyl linolenate, methyl palmitate, methyl stearate), monoglycerides (monoolein, monolinolenin, monostearin), diglycerides (distearin, dilinolenin), and triglycerides (tristearin, trilinolenin, trilinolein).

Sample and Standard Preparation

Biodiesel samples were obtained from students who had previously used the reactor to make fuel from used fry oil. Used fry oil samples were obtained from the Sagehen Café and new fry oil samples were obtained from Frank Dining Hall. All samples were dissolved in acetone to a concentration of 100 mg/mL. Standards were dissolved in either acetone or THF depending on their solubility. Stock solutions of either 100 mg/mL or 25 mg/mL of each standard were made. From these stock solutions, calibration standards of 10, 7, 5, 3, 1, and 0.5 mg/mL were made by serial dilution. Additional higher concentration standards were also prepared when necessary for

components with concentrations greater than 10 mg/mL. The portion of the sample or standard being analyzed was run through a Restek 0.45 μm PTFE syringe filter before being introduced to the HPLC.

Instrumentation

This method was developed on an Agilent 6120 Quadrupole LC/MS. Ionization for MS analysis was achieved through electrospray ionization (ESI). This instrument is also equipped with an Agilent 1200 series multiple wavelength detector (MWD), autosampler, thermostatted column compartment, degasser, and quaternary pump. Analyses were performed on an Agilent Eclipse XDB-C18 column, internal diameter 4.6 mm, length 150 mm, particle size 5 μm. Agilent ChemStation software was used to run analyses and perform peak integrations.

UV analyses were performed at a wavelength of 210 nanometers (nm) to ensure the transparency of the mobile phase solvents without exceeding the wavelength limit for analysis of FAMEs and glycerides. After about 220 nm, the UV spectrum of mixtures of FAMEs beings to decline[14]. The final separation conditions were arrived at by a series of trial and error runs of biodiesel and old fry oil samples. All subsequent analyses were performed under these conditions.

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Chapter 3 - Results and Discussion

Method Development

A variety of solvents (acetonitrile, methanol, acetone, and water) and their

mixtures were tried for the separation of the components of biodiesel. Separation

variables such as flow rate, column temperature, and injection volume were also varied to

determine the optimal separation conditions. Chromatograms were evaluated based on

peak resolution and time needed for all peaks to elute. Peak resolution refers to the degree

to which one peak is separated from another, and is usually measured by the distance

between two peaks at their base[15]. In general measures that tend to increase resolution

(such as decreased flow rates) also tend to increase total elution time[15]. The goal is to find

a solvent mixture and separation conditions that yield sufficient resolution while still

minimizing total elution time. The best separation was achieved using the solvents and

separation conditions listed in Table 2.

Table 2. Separation Conditions

Solvent A: 85% Acetonitrile/15% Water (0.1% TFA)
Solvent B: Acetone (0.1 % TFA)
Flow Rate: 0.7 mL/min
Injection Volume: 5 μL
Column Temperature: 50 °C

Mobile Phase Gradient		
Time (min)	Solvent A (%)	Solvent B (%)
0	100	0
10	100	0
20	70	30
40	20	80
50	0	100
60	0	100

Solvent Choice

Biodiesel, particularly poor quality biodiesel, may contain a wide variety of components including fatty acids, glycerides, and FAMEs. The polarity of these different groups of compounds covers a wide range and thus one solvent or solvent-mixture is not appropriate for separation. A solvent such as acetonitrile is appropriate for more polar compounds, such as fatty acids, but is not capable of efficiently moving non-polar compounds, such as triglycerides, through the column. Acetone is sufficiently non-polar to move triglycerides through the column, but causes fatty acids and monoglycerides to elute too quickly resulting in poor peak resolution. Many HPLC systems are now equipped with solvent delivery systems that allow for gradient elutions. These systems have two or more solvent inlet tubes. Solvent programs can be developed using the system software so that different proportions of the solvents are used throughout the
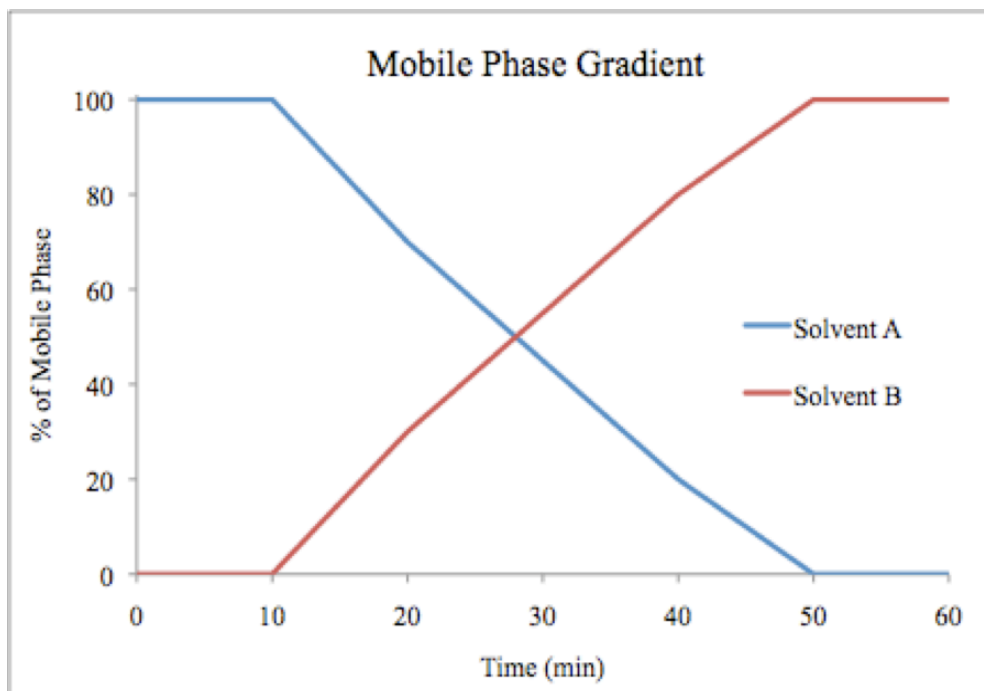
Figure 3. Shows the change in the composition of the mobile phase throughout the separation. Solvent A is 85% Acetonitrile 15% Water. Solvent B is Acetone. Both solvents contain 0.1% TFA.

separation. In this way you can begin your separation with a more polar solvent for more polar compounds, which elute first, and then move to a more non-polar solvent as increasingly non-polar components begin to elute.

This method begins with a mixture of 85% acetonitrile and 15% water as the initial solvent. This is the only solvent for the first 10 minutes. Acetone is then added to the solvent system in increasing proportions until it is the only solvent moving through the system at 50 minutes (Table 2, Figure 3). This allows the polar components to elute with sufficient resolution at the beginning of the separation without limiting elution of non-polar components towards the end of the separation.

Detection Methods

As mentioned earlier, UV detection requires the presence of a chromophore in the compound of interest. While many of the components of biodiesel have such chromophores, some do not and are therefore invisible to the UV detector. Of the standards used in this analysis, seven do not absorb in the UV: palmitic acid, monostearin, stearic acid, methyl palmitate, methyl stearate, distearin, and tristearin (Table 3). These compounds can only be detected and quantified using the mass spectrometer.

All compounds are theoretically detectable by the MS, which ionizes each compound as it elutes and measures the mass-to-charge ratio. The chromatogram produced by the MS is what is known as a total ion chromatogram (TIC). The detector measures the number of ions created by the ion source as the sample is eluted through the column. The number of ions is proportional to the concentration of each component. Normally, larger peaks indicate higher concentrations of a particular component.

However, the triglycerides did not exhibit this typical pattern. While the UV peaks of the

triglycerides grew larger with increasing concentrations, the TIC peaks grew smaller and

eventually became negative. This pattern can be observed by examining the

chromatograms of used fry oil, which is rich in triglycerides. Figure 4 shows three

different chromatograms of the used fry oil sample each run with a different injection

volume. A smaller injection volume is analogous to a lower concentration of the sample,

and vice versa. The smallest injection volume (1 µL) shows positive triglyceride peaks, as

would normally be expected. However, it is difficult to detect clear triglyceride peaks in

the chromatogram for the 3 µL injection volume. The first two peaks are smaller than the

1 µL injection volume and the remaining peaks are noisy. The chromatogram for the 5 µL

Table 3. Retention Times of Standards

Standard	Retention Time	
	UV	TIC
Monolinolenin	5.264 ± 0.019	5.246 ± 0.026
Linoleic Acid	8.716 ± 0.070	8.674 ± 0.036
Monoolein	9.976 ± 0.023	9.933 ± 0.064
Palmitic Acid	---	11.976 ± 0.069
Oleic Acid	12.741 ± 0.20	12.716 ± 0.19
Methyl Linolenate	13.474 ± 0.086	13.348 ± 0.031
Monostearin	---	15.531 ± 0.13
Methyl Linoleate	18.525 ± 0.060	18.427 ± 0.053
Stearic Acid	---	18.999 ± 0.16
Methyl Palmitate	---	22.892 ± 0.087
Methyl Oleate	23.446 ± 0.034	23.579 ± 0.12
Methyl Stearate	---	28.640 ± 0.093
Dilinolenin	31.668 ± 0.47	31.608 ± 0.46
Distearin	---	42.786 ± 0.096
Trilinolenin	43.829 ± 0.23	---
Trilinolein	46.976 ± 0.31	---
Tristearin	---	52.764 ± 0.61

Average retention times for UV peaks and TIC peaks are
given with 95% confidence intervals. "---" indicates no peaks
observed.

injection shows clear negative peaks for the triglycerides. These negative peaks are due to ion suppression. ESI has been shown to be particularly vulnerable to ion suppression compared to other ionization techniques[16]. While the mechanisms responsible for ion suppression are not yet fully understood, it has been observed that the characteristics and concentration of the analyte as well as matrix properties have an effect. High analyte concentrations result in competition for charge or space on the surface of ESI droplets. This inhibits of the ejection of ions trapped inside the droplet and results in ion suppression[17]. Ion-pairing agents, such as TFA, have also been shown to induce ion suppression in ESI-MS[18]. Both solvents used contained 0.1% TFA, so this likely contributed to the observed ion suppression.
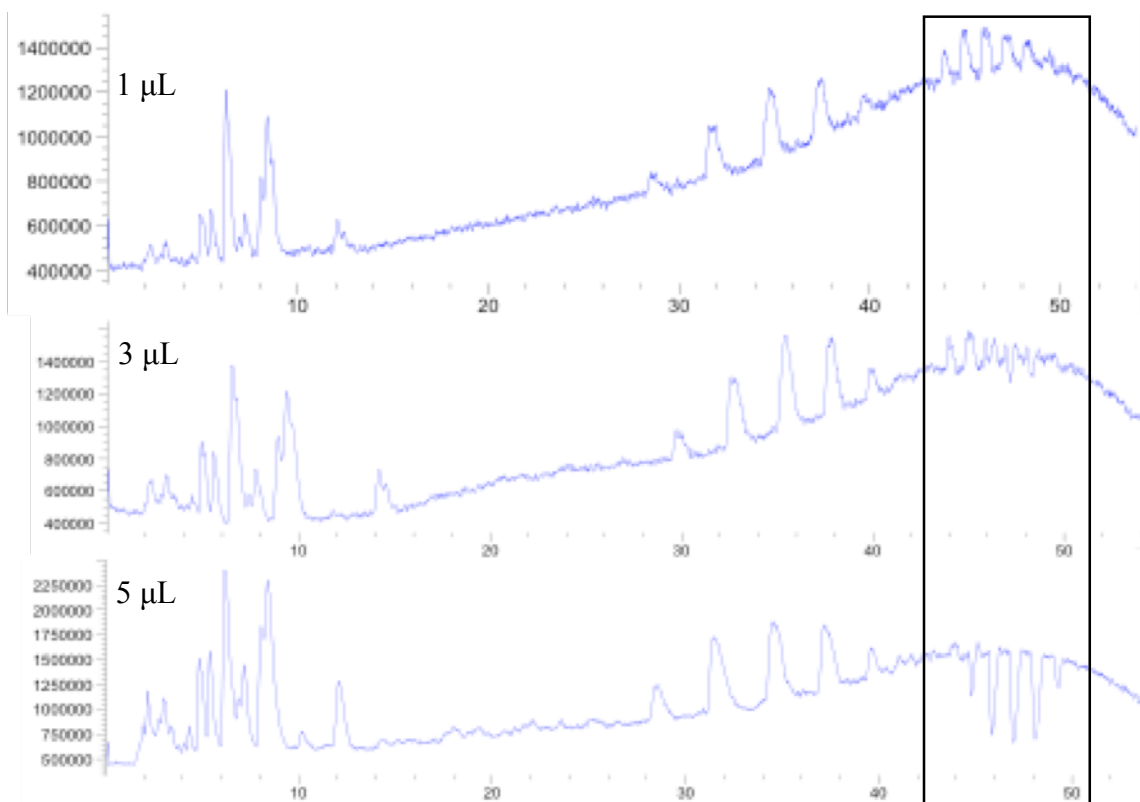
Figure 4. TIC chromatograms of used fry oil. All separation parameters constant except for injection volume: (top) 1 μL, (middle) 3 μL, and (bottom) 5 μL. Boxed area shows triglyceride peaks.

Peak Identification

Peaks were identified by comparing their retention times to those of known standards. Identification was confirmed by comparing the mass spectra of the peaks with those of the standards. Table 3 lists the retention times of all standards used. In general, monoglycerides and fatty acids eluted first, followed by FAMEs, then diglycerides, and finally triglycerides (Figure 5). This pattern is consistent with the literature[19-21]. The retention times for the peaks in the total ion chromatogram (TIC) are slightly smaller than those for the corresponding UV peaks. This is because the sample arrives at the mass spectrometer before it arrives at the UV detector, resulting in shorter retention times.

The identification of individual peaks was not possible in all situations. Due to time and financial constraints, it was not feasible to obtain and run standards for all potential components of the biodiesel mixture. Therefore, there are some peaks that do not correspond to any of the standards used, and thus cannot be positively identified based on retention time. Additionally, many peaks represent co-elutions. This means that there are two or more compounds that have very similar properties and elute at the same time. Each peak, therefore, does not necessarily represent just one compound. Since FAMEs are the main component of biodiesel, individual peak identification was focused on these chemical species (Figure 5).

The peak at around 13.9 minutes is methyl linolenate. Although the retention time of the methyl linolenate standard (13.348 min) is slightly lower than that of the corresponding peak in the biodiesel chromatogram (13.996 min), this is likely due to matrix effects rather than incorrect peak assignment. The mass spectra of the standard and the sample peak are similar and both have a large signal at $m/z = 293$. This

corresponds to the addition of H^+ to form the molecular ion (molecular weight of methyl linolenate = 292.46).

The peak at around 18.7 minutes is the co-elution of methyl linoleate and monostearin. The retention time of this peak (18.729 min) is very close to that of the methyl linoleate standard (18.427 min). The mass spectrum of the sample peak at 18.7 minutes has a large signal at $m/z = 245$, which corresponds with the mass spectrum of the methyl linoleate standard. The monostearin standard had a much lower retention time than this peak (15.531 min). This difference, however, is still likely due to matrix effects. The sample peak also had a large signal at $m/z = 381$, which matches with the mass spectrum of the monostearin standard. The co-elution of methyl linoleate and monostearin was also observed by Di Nicola et al. (2008). Monostearin does not absorb in the UV, so the UV signal is due only to the methyl linoleate.

The peak at around 24.2 minutes is the co-elution of methyl oleate and methyl palmitate. The methyl oleate (23.579 min) and methyl palmitate (22.892 min) standards both had retention times close that of this sample peak (24.255 min). Small differences can be attributed to matrix effects. The mass spectrum of the methyl oleate standard has a large signal at $m/z = 247$. This signal was also present in the mass spectrum of the sample peak at 24.2 minutes. The sample peak also had a large signal at $m/z = 271$, which matches with the mass spectrum of the methyl palmitate standard. The co-elution of methyl oleate and methyl palmitate was also observed by Di Nicola et al. (2008). Methyl palmitate does not absorb in the UV, so the UV signal is due only to the methyl oleate.
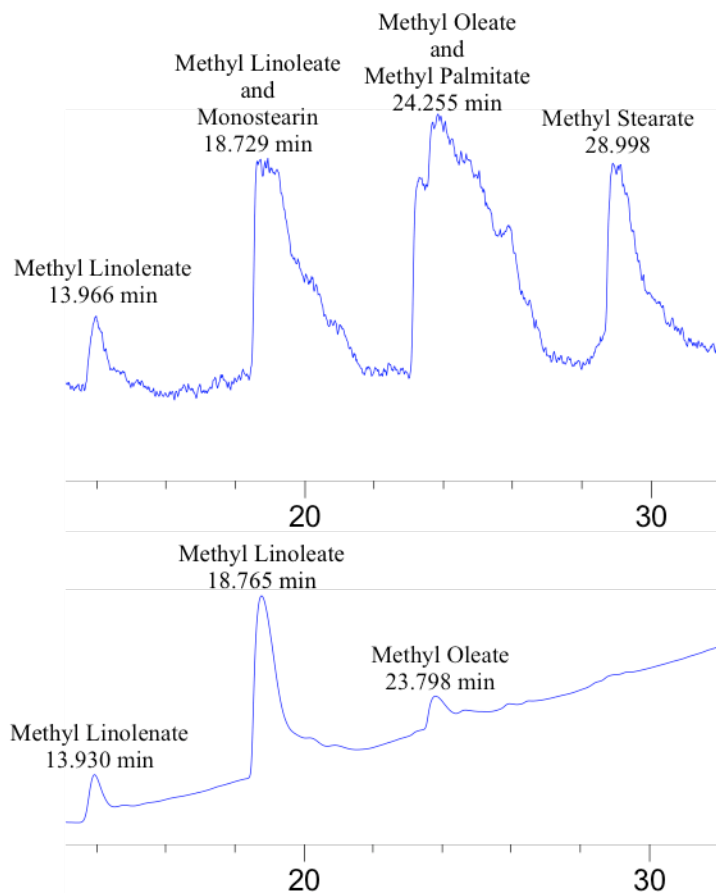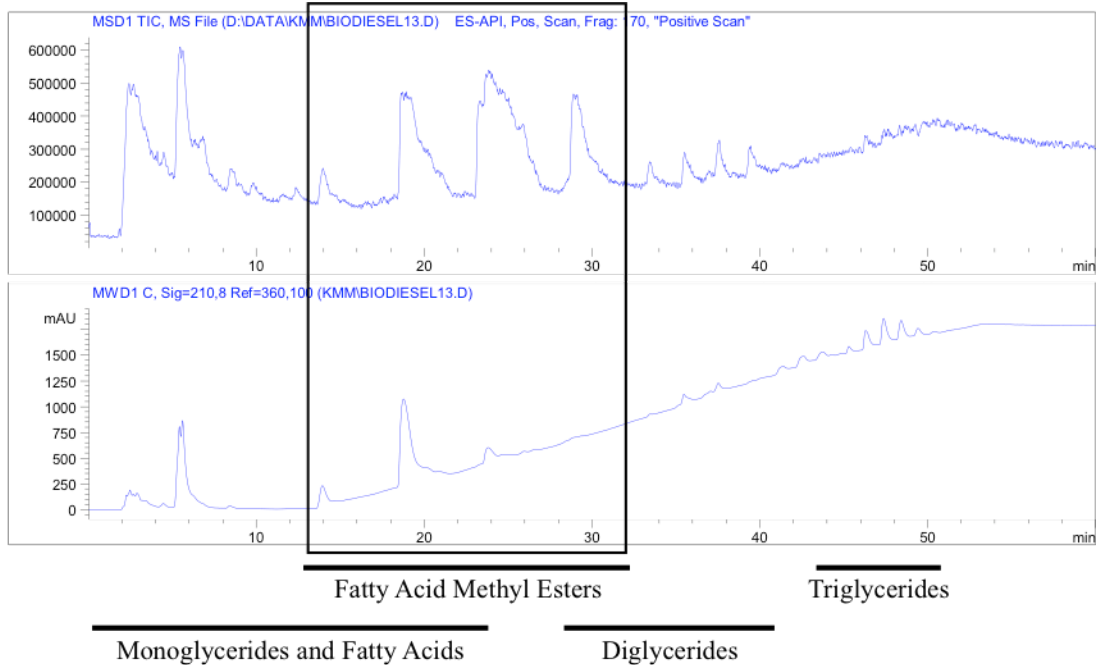
Figure 5. Total ion (top) and UV (bottom) chromatograms of biodiesel. Run under conditions listed in Table 2. Boxed area enlarged to show identification of FAME peaks.

The last peak, at around 29 minutes is methyl stearate. The retention time of this peak (28.998 min) is very close to that of the methyl stearate standard (28.640 min). Again, small differences are likely due to matrix effects. The mass spectrum of this sample peak has a large signal at $m/z = 299$, which agrees with the mass spectrum of the methyl stearate standard. Additionally, there is no UV signal, which is consistent with methyl stearate, which has no chromophore and thus does not absorb in the UV. Di Nicola et al. (2008) found that methyl stearate and dilinolenin co-elute. This was not observed here because there is no dilinolenin in this sample. Dilinolenin absorbs in the UV, but there is no corresponding UV peak indicating its presence. Additionally, the mass spectrum of the dilinolenin standard has a large signal at $m/z = 615$. The mass spectra of this peak and the subsequent diglyceride peaks all lack this signal, suggesting there is no dilinolenin present in this sample. It is important to note, however, other biodiesel samples may have dilinolenin and it will likely co-elute with the methyl stearate. The presence of a corresponding UV peak will indicate that there is dilinolenin in the sample.

Peak Quantification

The ASTM standards provide limits on the amount of glycerol related compounds (i.e. free glycerol, monoglycerides, diglycerides, and triglycerides) allowed in the biodiesel. However, it is difficult to directly measure these compounds. A large number of standards would be required to cover all possible components of the mixture and each peak would have to be individually quantified. Additionally, these compounds will be present in very low concentrations in quality biodiesel. This causes a problem because quantification becomes less accurate, or even impossible, at low concentrations near the

detection limit. The quantification of triglycerides would be particularly difficult because of the ion suppression problems mentioned above. FAMEs, however, will be present in high concentrations and require only a few standards for quantification. Aside from the possibility of residual methanol, the range of possible compounds in the biodiesel mixture is limited to glycerol related compounds, free fatty acids, and FAMEs. ASTM requires the amount of free glycerol to be less than 0.02% (w/w) and the amount of bound glycerol to be less than 0.24% (w/w)[1]. There is no published limit on the amount of allowed free fatty acids, so we will assume high quality biodiesel is fully converted and should not contain any fatty acids (0%). Therefore, the biodiesel should be at least 99.74% (w/w) FAMEs. Using this value to assess the fuel quality allows us to limit quantification to FAME peaks.

Table 5. Concentration of Methyl Esters in Biodiesel Sample

FAME	Detection Method	Peak Area	Concentration (mg/mL)
Methyl Linolenate	MS	5204250	2.55
	UV	5902 mAU*s	0.83
Methyl Linoleate	UV	44360 mAU*s	13.92
Methyl Oleate	UV	6600 mAU*s	16.31
Methyl Palmitate	EIC	2020470	1.48
Methyl Stearate	MS	23181600	59.64
TOTAL			**93.90**

The methyl linolenate peak (at around 13.9 minutes) was quantified based on calibration curves made from both UV and TIC peak areas (Figure 6). Based on the TIC calibration, the concentration of methyl linolenate is 2.55 mg/mL (Table 5). Based on the UV calibration, the concentration of methyl linolenate is 0.83 mg/mL (Table 5). The difference between these two values is likely due to errors in peak integration. The UV
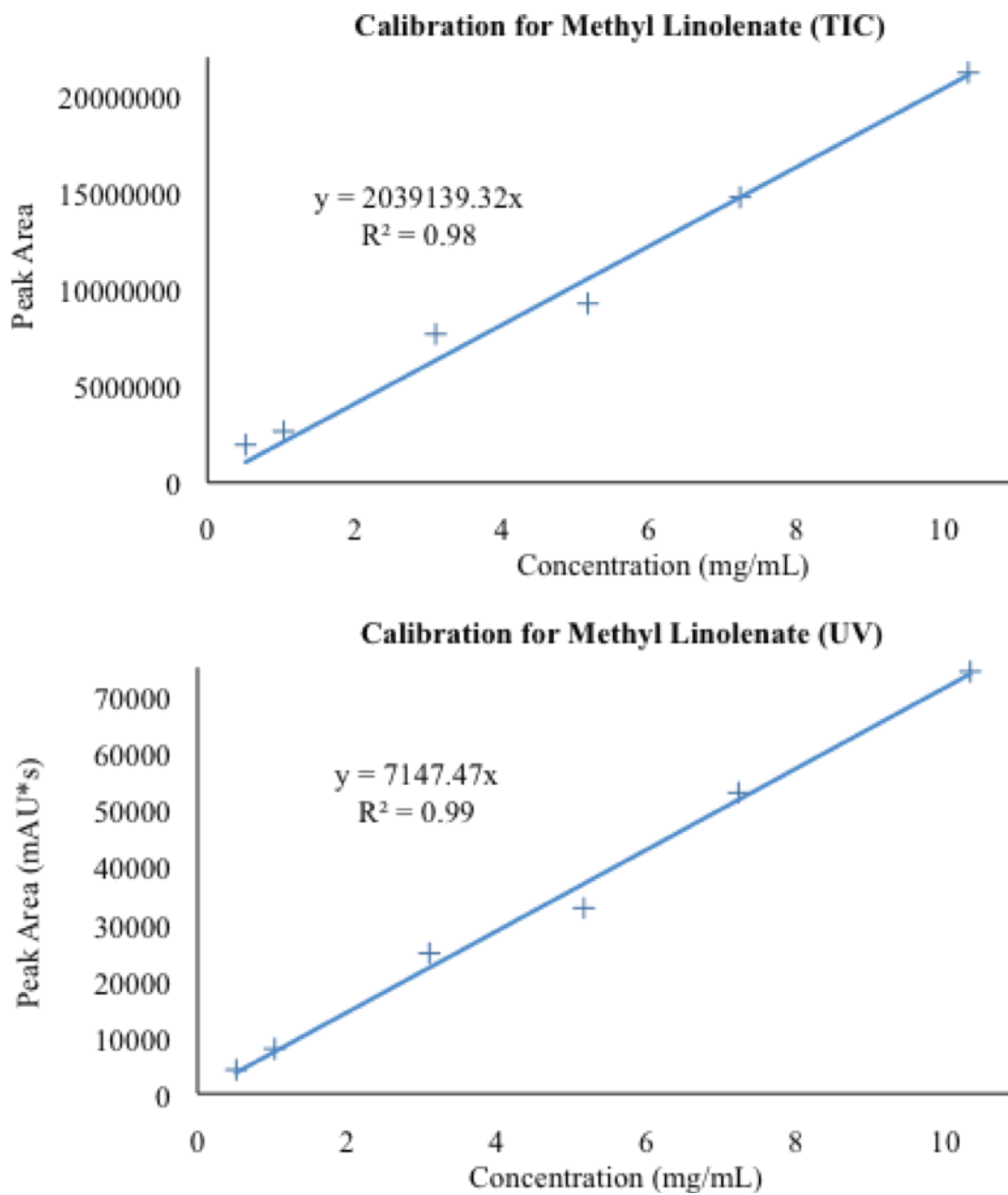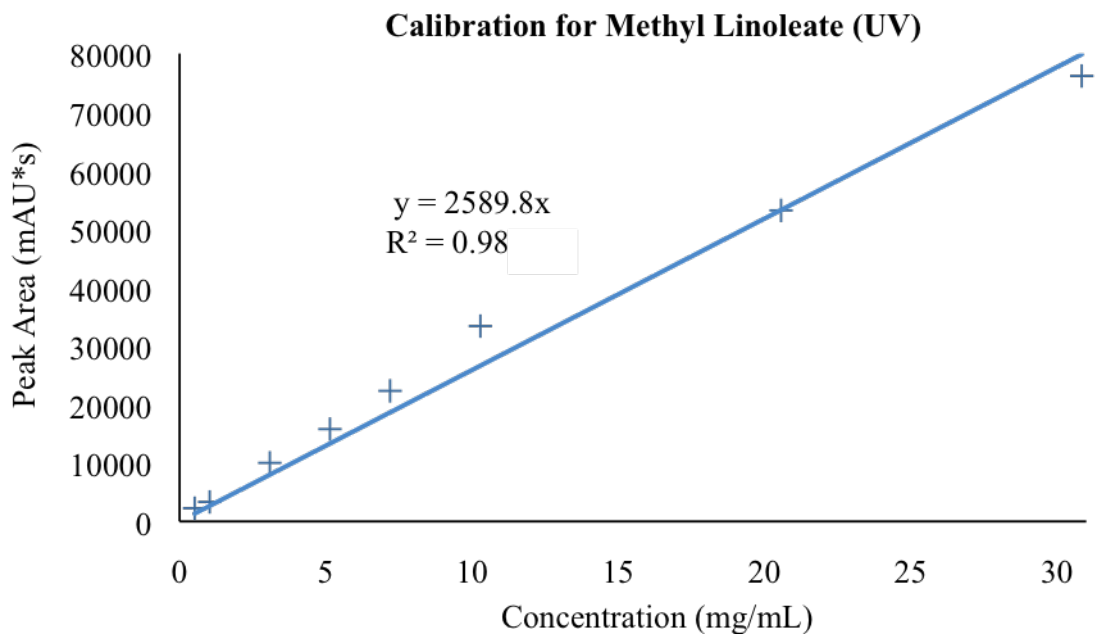
Figure 6. Calibration curves for methyl linoleate based on TIC (top) and UV (bottom) peak areas.

peaks are much smaller than the TIC peaks so small errors in integration have a much larger effect. Additionally, this peak is located at the point where the baseline in the UV chromatogram begins to rise rapidly due to changes in solvent composition during the gradient elution (Figure 5). Rising baselines make peak integration more complicated and vulnerable to error. Since the TIC peaks are bigger and experience less dramatic baseline rise, it is likely that the TIC peak area is more accurate than the UV peak area and the true concentration is closer to 2.55 mg/mL.

The peak at about 18.7 minutes contains two components, methyl linoleate and monostearin. The TIC peak area tells us little about their individual concentrations since both compounds contribute to the number of total ions. Monostearin, however, does not absorb in the UV. The UV peak area can therefore be used to quantify methyl linoleate. The area of the UV peak = 44360 mAU*s and the concentration of methyl linoleate in the sample = 13.92 mg/mL (Table 5, Figure 7).
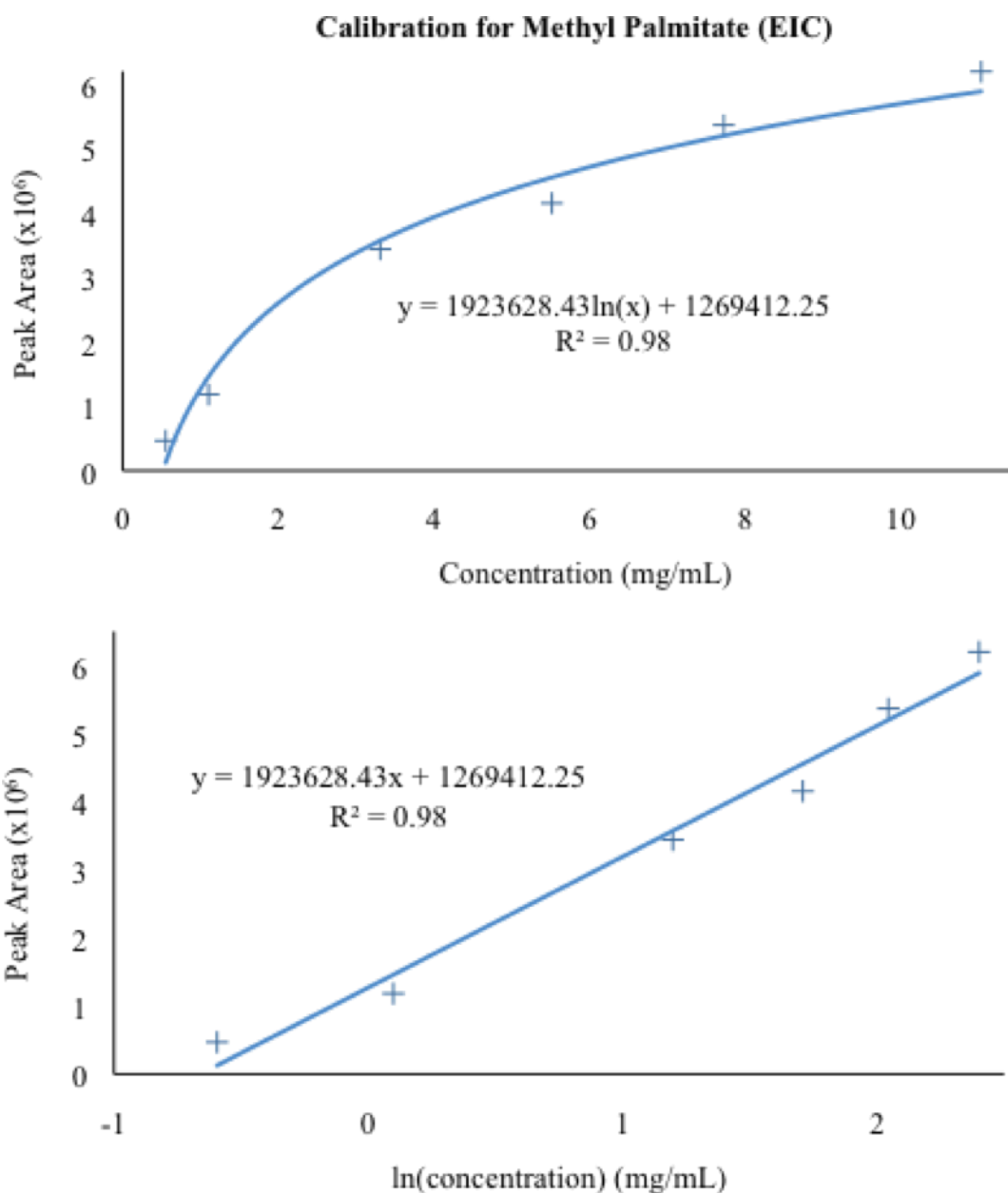

Figure 7. Calibration curve for methyl linoleate based on UV peaks.

The peak at about 24.2 minutes also contains two components, methyl oleate and methyl palmitate. Methyl palmitate does not absorb in the UV, so the UV peak area can be used to determine the methyl oleate concentration. The area of the UV peak = 6600 mAU*s and the concentration of methyl oleate in the sample = 16.31 mg/mL (Table 5, Figure 8).

Calibration for Methyl Oleate (UV)

$y = 404.74x$
$R^2 = 0.99$

Peak Area (mAU*s) vs Concentration (mg/mL)
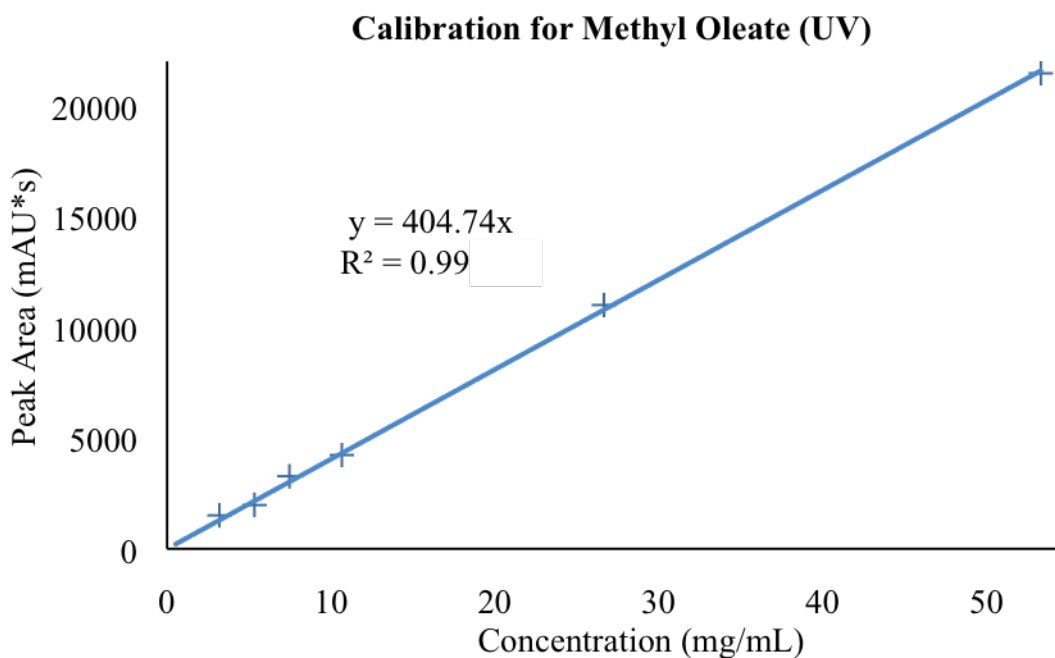
Figure 8. Calibration curve for methyl oleate based on UV peak areas.

The methyl palmitate concentration cannot be determined using the area of the TIC peak since only some of the ions are due to methyl palmitate. It is, however, possible to generate extracted ion chromatograms (EIC), which measure the abundance of one particular ion at a specified mass-to-charge ratio. The mass spectrum of the methyl palmitate standard shows a large signal at $m/z = 271$. The abundance of this ion is proportional to the concentration of methyl palmitate. By generating an EIC at this m/z value for each standard, it is possible to create a calibration curve for just that ion (Figure

Figure 9. Calibration curve for methyl palmitate based on EIC peak areas (top) and linearized calibration of EIC peak area vs ln(concentration) (bottom). EIC taken at *m/z* = 271.
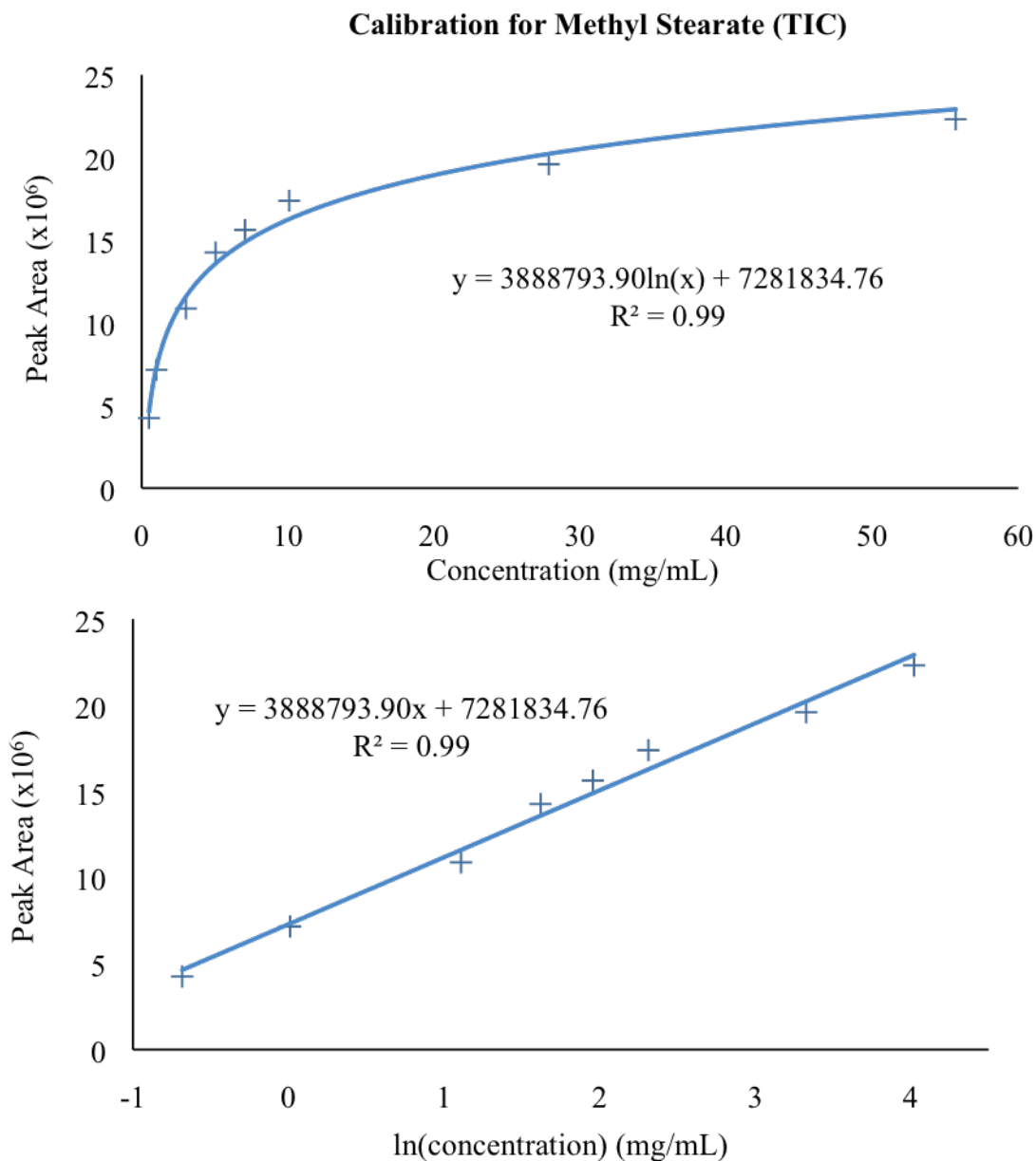
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9). The EIC chromatogram at *m/z* = 271 for the biodiesel shows one peak around 23 minutes with an area of 2020470 (Figure 10). This corresponds to a methyl palmitate concentration of 1.48 mg/mL (Table 5). This value should be used with caution because it is beyond the linear range for the methyl palmitate calibration and more prone to error.

The final FAME peak at around 29 minutes contains just methyl stearate. The TIC peak area is 23181600 and the concentration of methyl stearate in the sample is 59.64 mg/mL (Table 5, Figure 11). This value should be used with caution because it is beyond the linear range for the methyl stearate calibration and more prone to error. Methyl stearate does not absorb in the UV, so there is no UV peak.
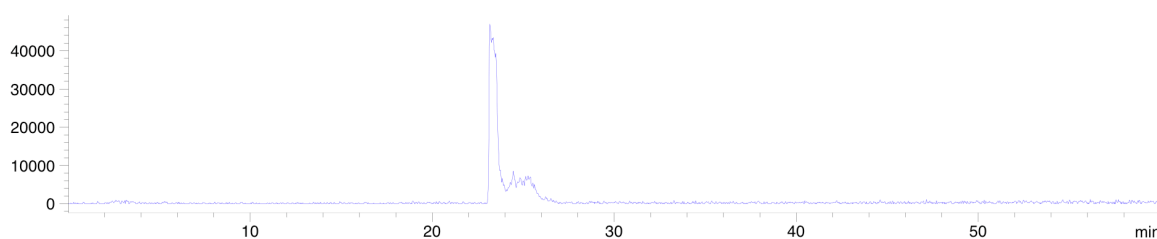
Figure 10. Extracted ion chromatogram for biodiesel at *m/z* = 271.

The total FAME concentration can be calculated by adding the concentrations of the individual methyl esters. This biodiesel sample has a FAME concentration of 93.90 mg/mL (Table 5). The biodiesel sample used is a 100 mg/mL dilution in acetone, so the sample is 93.90% FAME (w/w). This does not meet the minimum 99.74% required by the ASTM standard. The chromatogram shows some small peaks indicating the presence of diglycerides and triglycerides, suggesting that the reaction was not allowed to go to completion, leaving these unreacted contaminants. The biggest non-FAME peaks, however, fall into the fatty acid and monoglyceride region. While monoglycerides are also the result of an incomplete reaction, the presence of fatty acids suggests that not enough catalyst was used. The fatty acid concentration of the vegetable oil starting

Calibration for Methyl Stearate (TIC)

$$y = 3888793.90\ln(x) + 7281834.76$$
$$R^2 = 0.99$$

$$y = 3888793.90x + 7281834.76$$
$$R^2 = 0.99$$

Figure 11. Calibration curve for methyl stearate based on TIC peak areas (top) and linearized calibration of TIC peak area vs. ln(concentration) (bottom).

material can vary widely, especially when waste vegetable oil is used. A preliminary titration of the oil can be performed to determine the acid content. This information can then be used to determine the appropriate amount of catalyst so that all the fatty acids are neutralized. It is likely that a preliminary titration was not performed for this sample, or was done incorrectly. In order to improve the biodiesel quality in the future, a titration

should be performed to determine the correct amount of catalyst. The reaction mixture should be allowed to sit in the reaction tank for a longer period of time to ensure a complete reaction.

Chapter 4 - Conclusions

The separation and quantification of the components of biodiesel was successfully achieved using a gradient reverse-phase HPLC method utilizing UV and MS detection. By limiting identification and quantification to peaks containing FAMEs, it is possible to assess the quality of the biodiesel without the need for a large number of standards with individual calibrations to cover all possible components of the biodiesel. It is likely that in high quality biodiesel contaminant peaks will be so small that preliminary qualitative analysis will be possible to assess the quality of the fuel. Since 99.74% of the biodiesel should be FAMEs, those peaks should be much larger than any others in the chromatogram. A sample analyzed using this method that yields four large peaks between 13 and 29 minutes is indicative of quality biodiesel. Any other peaks that are present should be very small in comparison and few in number. While monostearin and dilinolenin in the sample would co-elute with the FAME peaks, the FAMEs they elute with do not absorb in the UV. Their existence can therefore be detected by the presence of a corresponding UV peak. This allows for the detection of artificially large FAME peaks due to co-eluting contaminants, reducing the possibility of mistaking poor quality fuel for high quality fuel.

This method allows for the relatively cheap analysis of biodiesel made in the Appleseed reactor on campus because it utilizes instrumentation and materials that are available in Pomona College's Chemistry Department. Additionally, the procedure requires very little sample preparation and is relatively safe, as long as general lab safety practices are followed. After some general instruction in how to use the HPLC

instrumentation and software, even someone without a background in chemistry would be able to perform this procedure and test the quality of their biodiesel.

Analysis of the biodiesel chromatograms can also be used to optimize the procedure used to make the biodiesel. Different types of contaminants in the fuel point to different problems in the production procedure. If many fatty acids are present, it is likely that too little catalyst was used. If there are many mono-, di-, and triglycerides, the reaction was probably not allowed to go to completion. By knowing what steps went wrong, students will be able to correct mistakes and develop a procedure that produces high quality biodiesel. An optimized production procedure and a test method to assess the final product will ensure high quality fuel that can be used with confidence in diesel engines.

The use of this procedure will add strength to proposals to increase the use of the Appleseed reactor and produce biodiesel for campus grounds equipment from waste vegetable oil. This will reduce fuel costs for the college and lower CO_2 and other air pollutant emissions. It also offers a way to recycle the used fry oil from dining halls and other campus eating establishments, which will cut down on waste disposal costs. If it can be proven that the fuel produced by the reactor will not harm the engines, it is more likely the college will agree to its use in grounds equipment.

Acknowledgments

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