# Forensic analysis of biodiesel

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ABSTRACT

The analysis of four different biodiesel blends, as well as homemade biodiesel prepared from vegetable oil, has been performed using gas chromatography-mass spectrometry. The identification of methyl esters within the biodiesel along with any background components is made possible by recognizing their mass spectral fragmentation patterns. These fuels were subjected to typical fire scene environments, specifically weathering and microbial degradation, to investigate how these environments affect the analysis. A matrix study was also performed on wood, carpet, and clothing in order to identify any interferences from these substrates. The data obtained herein will provide the forensic science community with the data needed to help recognize these increasingly common ignitable liquids.

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## 1. Introduction

The use of alternative fuels has increased dramatically over the last several years, as more people look for ways to save money, reduce environmentally harmful emissions, and decrease their dependence on fossil fuels. Specifically, biodiesel made from either waste vegetable oil (WVO) or straight vegetable oil (SVO) has become popular since it is inexpensive, readily available, and renewable. In 2014, the United States biodiesel industry produced over 1.27 billion gallons of biodiesel [1]; in Europe over 7 billion gallons were produced [2]. Most of this biodiesel is produced from some kind of vegetable oil (soybean and canola oils are the most commonly used feedstocks). There are currently 120 plants in the United States that are members of the National Biodiesel Board [3], and over 50 members of the European Biodiesel Board [2]. In addition, more people are making biodiesel in their homes, using inexpensive WVO or SVO and other household materials. Typically these materials include methanol and a strong base such as sodium hydroxide or potassium hydroxide, all of which are easy to obtain in large quantities. The reaction is a straightforward transester-ification of triacylglycerides (fats) to fatty acid methyl esters (FAMEs, Fig. 1), but involves the use of corrosive and flammable materials.

Because of the increasing use and manufacture of biodiesel, both commercially and in people's homes, it is expected that these fuels will be present in increasing numbers of submissions to fire debris analysts. Many people who make biodiesel at home are working with several gallons of fuel at a time. Many recipes for making biodiesel at home can be found very easily on the Internet; an online search for "make biodiesel at home" returned over one million results [4]. Consequently, many people who are relatively inexperienced at working with corrosive and flammable materials in large quantities are doing just that, and the potential for fires and explosions is high. There are already several reports of fires starting at homes and at biodiesel processing plants, where large amounts of methanol, oils, and strong bases are stored and heated [5]. These fires have led to hundreds of thousands of dollars in damage. Because of this, fire investigators and fire debris analysts will be encountering biodiesel in more routine casework.

While analysts are comprehensively trained in identifying a wide variety of petroleum-based products, it is relatively rare to see non-petroleum based samples (e.g., oxygenated products and terpenes). Currently, there is little training or experience with biofuels. This point was made in 2007 by Stauffer and Byron in their excellent introduction to biodiesel for fire debris analysts [6]. These authors addressed the basic analytical profiles of biodiesel and noted several considerations that are unique to biodiesel and vegetable oil, such as aging effects and decreased volatility. Since this introduction, there has not been a thorough study of the analysis of biodiesel. The effects of weathering, degradation, and substrate involvement have been systemati-cally studied for petroleum products [7,8], but these and other effects have not been examined with respect to the analysis of biofuels.

Herein we report what is to the best of our knowledge the most complete forensic analysis of biodiesel to date. Following

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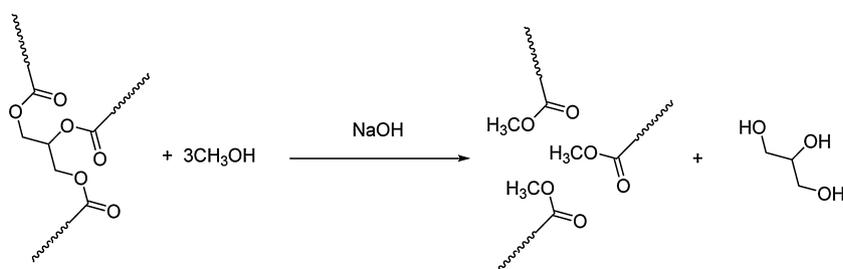  E-mail address: finneyee@plu.edu (E.E. Finney).

**Fig. 1.** General reaction scheme of the production of FAMEs that make up biodiesel from a triacylglycerol. Glycerol is the other product formed in the reaction.

modifications of the procedures set by ASTM international [9] which are commonly used in routine fire debris analysis, we set out to understand the forensic aspects of biodiesel at the same level as our current understanding of petroleum products. Specifically, we have studied: (i) neat liquid samples of homemade biodiesel along with commercial samples of B5, B20, B50, and B99 blends, as compared to regular diesel fuel; (ii) heated-headspace extraction samples of each type of biodiesel, at two different temperatures; (iii) the effects of microbial degradation on analysis; (iv) the effects of evaporation on analysis; and (v) the potential for matrix effects to interfere with the identification of biodiesel. We also examined the differences encountered when biodiesel is made using different types of oils. It is our goal to provide the forensic science community with as complete a profile as possible of a wide range of biodiesel products. We predict that the increasing use of biofuels and subsequent increase in their appearance in fire debris will eventually warrant the formation of a new class of ignitable liquids [10], or at least a sub-classification within oxygenated products.

We have chosen to focus on the analysis of the B20 biodiesel blend herein for two reasons. First, this blend is the most commonly used, at least in the United States [11]. Second, chromatographically it is the most interesting blend because it contains significant amounts of both diesel and FAMEs. This makes B20 ideal for studying the differences of the behavior of biodiesel compared to well-known petroleum-based (especially diesel) samples.

## 2. Materials and methods

### 2.1. Materials

Carbon disulfide (ACS grade) and 1-chlorohexadecane (99%) were purchased from Aldrich and used as received. A stock solution containing 0.1% 1-chlorohexadecane in $CS_2$ was prepared and used to elute the charcoal strips when comparisons to an internal standard were desired. Methanol (ACS grade), dichloromethane (99%), and sodium hydroxide were purchased from Fisher Scientific. Unlined metal paint cans were purchased from House of Cans (www.houseofcans.com). Activated charcoal strips (8 × 20 × 1 mm) were purchased from Albrayco Technologies. Heet® (a methanol-based fuel additive), Drano® (an NaOH-based drain cleaner), and Miracle-Gro® potting soil were purchased at local hardware stores and used as-is.

Homemade biodiesel was prepared from commercial vegetable oil using a common, publicly available procedure [12]. Briefly, 3.52 g of Drano® professional strength crystals was added to 210 mL of Heet®. This mixture was shaken to dissolve the crystals, and the metal shavings from the Drano® were filtered. This solution was slowly added to 1 L of new vegetable oil and the solution was heated to 55 °C with stirring. After stirring for 20 min, the mixture was left overnight to allow two layers to separate. The

top biodiesel layer was removed in 300 mL portions and each portion was washed three times with 100 mL of deionized water. The biodiesel layers were combined and heated to 100 °C to remove any remaining water, and the final product was characterized by infrared spectroscopy and nuclear magnetic spectroscopy. Spectral data of the final product are available in the Supplementary Information.

Biodiesel was also prepared from coconut, safflower, peanut, and cottonseed oils on a smaller scale using methanol and sodium hydroxide instead of Heet® and Drano®; the experimental procedure was the same as that described above.

Commercial biodiesel samples (B5, B20, B50, and B99) were obtained from various fueling stations in the Seattle, WA area.

### 2.2. Instrumentation

GC–MS data were collected using a Hewlett-Packard 6890 gas chromatograph with a 5973 mass selective detector (MSD). The MSD was operated in electron ionization mode with an ionization potential of 70 eV and a scan range of 50–550 amu. The ion source temperature was maintained at 230 °C.

Chromatographic separation was carried out using a capillary column Restek Rxi-35Sil (30 m × 0.25 mm × 0.25 μm). The GC was operated at a 50:1 split ratio (1 μL injection volume) with a constant helium flow of 1.0 mL/min. The GC injector temperature and transfer line were both maintained at 280 °C. The oven temperature was programmed as follows: initial temperature, 60 °C, held for 2 min; ramp rate, 10 °C/min to 200 °C, 5 °C/min to 240 °C, held for 6 min; total run time 30 min.

### 2.3. Methods

#### 2.3.1. Analysis of neat samples
Neat samples were analyzed by placing 10 μL of biodiesel fuel in a 1.5 mL gas chromatography vial. Carbon disulfide (1.5 mL) was added to the vial and the sample was analyzed by GC–MS.

#### 2.3.2. Heated headspace analysis: General procedure
The material to be analyzed was placed in an unlined metal can. An activated charcoal strip was suspended in the can's headspace using an alligator clip inside the can lid held in place with a magnet on the outside of the lid. The can was sealed and placed in an oven set at either 75 °C or 100 °C for 6 h. The can was allowed to cool to room temperature and the charcoal strip was removed. The strip was placed in a GC vial and eluted with 1.5 mL of $CS_2$. This sample was analyzed by GC–MS.

#### 2.3.3. Preparation of samples for microbial degradation
For each sample, approximately 50 g of potting soil was added to a can. Approximately 1 mL of the biodiesel sample was added to the soil and the can was sealed for 2, 7, 14, 30, or 60 days at room temperature. After that time, the can with the sample inside was

analyzed using the heated-headspace procedure described above. In addition to the timed samples, a set of control samples was prepared in which the cans containing dirt and fuel were subjected to headspace analysis immediately after adding the fuel. The carbon strip from each sample was analyzed twice; after analysis in $CS_2$, the solvent was evaporated and 1.5 mL of $CS_2$ containing 0.1% 1-chlorohexadecane as an internal standard was added.
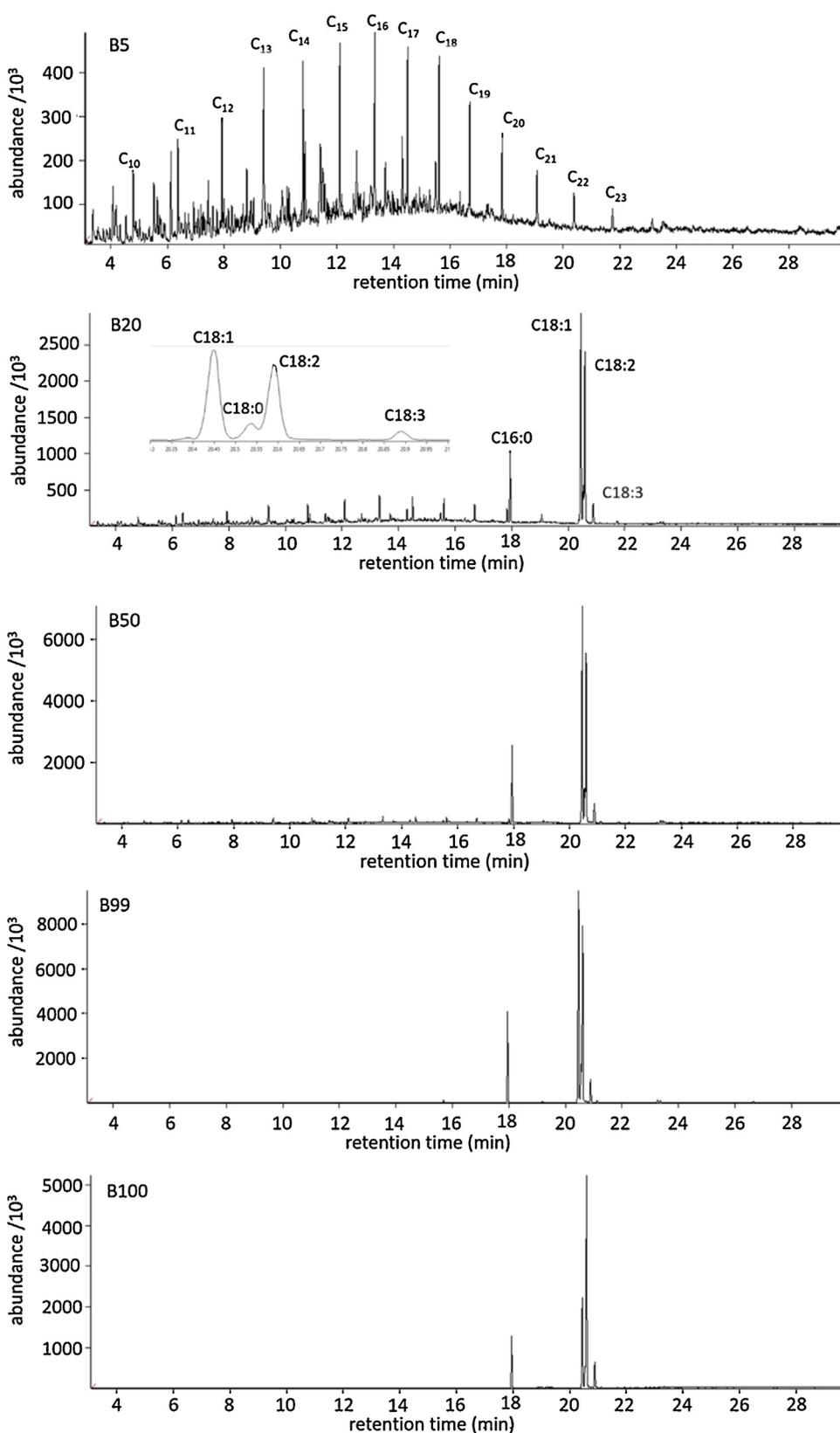


Fig. 2. Total ion chromatograms of the neat samples of (from top to bottom) diesel fuel, B5, B20, B50, and B99 biodiesel blends, and homemade biodiesel (B100).

### 2.3.4. Evaporation studies

A 100-mL sample of each fuel was placed in a beaker and set in a fume hood. The samples were gently heated and a stream of air was applied to aid in evaporation. Aliquots of 10 μL were removed after the volume of the fuel had been reduced to 75 mL, 50 mL, and 25 mL (i.e., to 25%, 50%, and 75% evaporation). Heating was applied to all of the samples to force evaporation to their fullest extent. The B5 and B20 fuels evaporated to 5 mL (95%), the B50 fuel evaporated to 15 mL (85%), and the B99 and B100 (homemade) fuels stopped evaporating at 25 mL (75%). All of the fuels became black, tarry material after they had evaporated to their fullest extent.

### 2.3.5. Matrix samples

Burned and unburned samples of wood (cedar lumber and a pine tree branch), carpet, and clothing (cotton and polyester) in the absence of any fuel were analyzed according to the general procedure described above.

## 3. Results and discussion

### 3.1. Analysis of neat samples

The total ion chromatograms of neat biodiesel blends, along with diesel fuel and homemade biodiesel from new vegetable oil, are shown in Fig. 2. Under the analysis method described above, no peaks from the regular vegetable oil were observed in the chromatogram, but the FAME products of the transesterification reaction were observed. Notably, no FAME peaks were observed in the B5 sample at all, even when viewing the extracted ion chromatogram (see below). Under the conditions used herein, the B5 fuel was essentially identical to standard diesel fuel. The FAME peaks were observed during the evaporation tests for this fuel, as will be seen later.

For the other biodiesel blends, the usual Gaussian set of peaks characteristic of a heavy petroleum distillate is immediately recognizable, and is augmented by the presence of four major FAME peaks. As expected, the relative abundance of the diesel peaks decreases with increasing proportion of FAMEs. The FAME peaks present in the biodiesel samples are identified in the chromatogram. For the biodiesel made from canola oil used in this study, the primary FAMEs present are of $C_{16}$ and $C_{18}$ fatty acids. The $C_{16}$ ester is saturated methyl hexadecanoate (palmitate, C16:0 [13]). The $C_{18}$ esters are comprised of the saturated methyl stearate (C18:0) along with monounsaturated (methyl oleate, C18:1) and di-unsaturated (primarily methyl linoleate, 9,12-octadecadieno-ate, C18:2).

It is important to note that while biodiesel is typically made using vegetable oil (either new or used), other oils have been used to make biodiesel, and the composition of the oil used to make the fuel will determine the appearance of the chromatogram. Therefore, we also examined the products of transesterification reactions of coconut, safflower, peanut, and cottonseed oils. Of these, all except for coconut oil contained the same FAMEs as canola oil-derived biodiesel, but in different ratios (see the Supplementary Information for chromatograms). The peanut oil-derived sample contained longer-chain FAME components as well (up to C22:0, methyl behenate). The coconut oil chromatogram, shown in Fig. 3, shows a regular series of saturated methyl esters starting with a small peak corresponding to hexanoate (caproate), and increasing by two carbons up to hexadecanoate, after which small amounts of unsaturated $C_{18}$ esters appear. Because biodiesel prepared from these oils is not as common as that prepared from canola oil, these biodiesel products are not examined further in the present report.

### 3.2. Headspace extraction

To see how the chromatograms change when the heated headspace of the biodiesel blends are sampled, we performed control extractions in which a 1.0 mL sample of liquid was placed in a sealed can and extracted onto an activated charcoal strip. We began the control extractions using a standard temperature of 75 °C; the resulting chromatograms are shown in Fig. 4. Immediately noticeable is that FAME peaks diminish significantly relative to the hydrocarbon peaks when headspace extraction is performed, due to their higher boiling points.

Since liquid samples are relatively rarely encountered in casework, and heated headspace techniques are commonly used to analyze fire debris samples, we decided to extract the samples in the sealed cans at higher temperatures to see what differences would be seen between diesel and the biodiesel blends. Heating new samples at 100 °C gave the chromatograms shown in Fig. 5. As expected, the higher-temperature extraction favors the higher-boiling components of each fuel, which in the case of biodiesel are mostly heavier FAMEs in addition to heavier hydrocarbons. In addition, a greater amount of material in general was adsorbed onto the charcoal strips at 100 °C, as indicated by the increase in abundances in the chromatograms. The adsorption of the FAME components in particular increases by an order of magnitude when the analysis is performed at 100 °C vs. 75 °C.

The behavior of the homemade B100 was different from that of the blends with lower fractions of biofuel when analyzed at high temperature. While the headspace sample heated to 75 °C showed the expected FAME peaks and no other compounds, the sample analyzed at 100 °C showed a number of small oxygenated compounds (compare Figs. 4 and 5). These compounds, summarized in Table 1, are known to result from the oxidation of biodiesel by molecular oxygen over time [14,15]. A mechanism for the
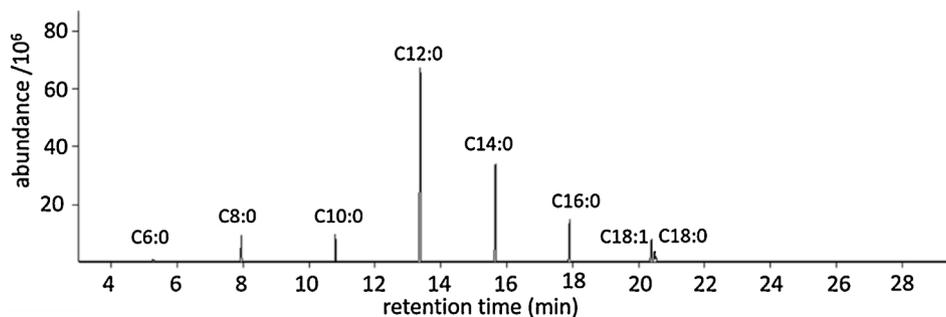


**Fig. 3.** Total ion chromatogram of homemade biodiesel prepared from coconut oil.
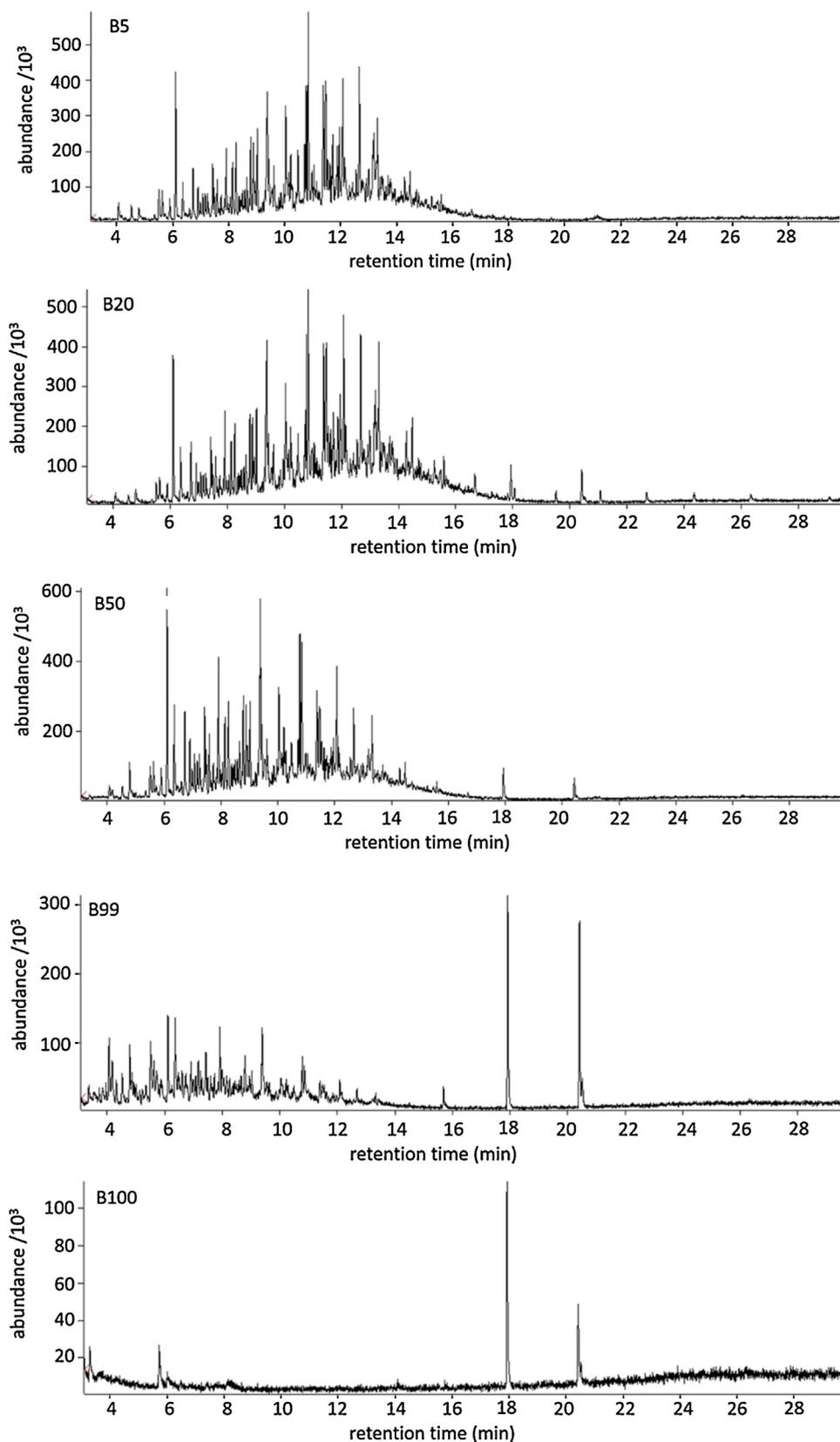
**Fig. 4.** Total ion chromatograms of (from top to bottom) diesel, B5, B20, B75, B99, and homemade biodiesel (B100) analyzed using heated headspace sampling at 75 °C.

formation of 2-pentylfuran has been proposed [16]; that mechanism as well as proposed mechanisms for the formation of the other compounds in Table 1 are given in the Supplemental Information. These compounds were not observed in the 100 °C

analysis of the B5, B20, or B50 fuels, but were seen in the weathered B99 samples (Supplementary Information). It should be noted that small-chain aldehydes, substituted furans, and related compounds have also been observed in burned clothing and paper,
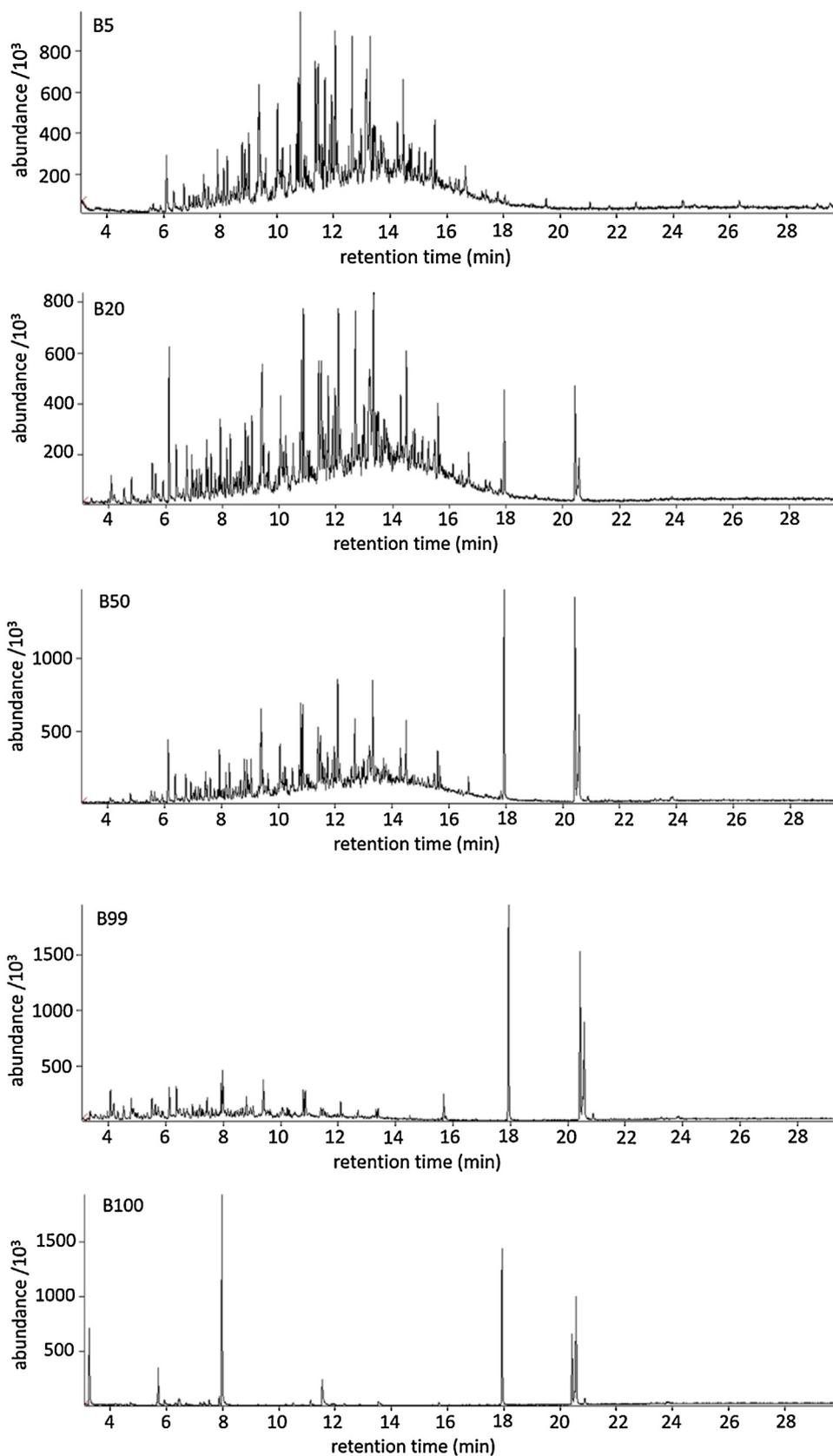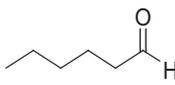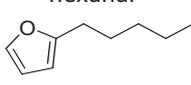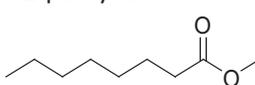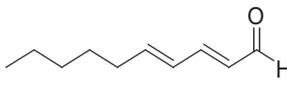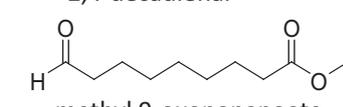
**Fig. 5.** Total ion chromatograms of (from top to bottom) diesel, B5, B20, B75, B99, and homemade biodiesel (B100) analyzed using heated headspace sampling at 100 °C.

**Table 1**
The major oxygenated decomposition products of B100 observed from the heated-headspace sample at 100 °C.

| Retention time (min) | Compound |
|---|---|
| 3.25 | hexanal |
| 5.70 | 2-pentylfuran |
| 7.97 | methyl octanoate |
| 11.54 | 2,4-decadienal |
| 13.55 | methyl 9-oxononanoate |

**Scheme 1.** Common fragmentation routes of methyl esters giving rise to $m/z$ 59 and $m/z = 74$ ions in the mass spectra.

so that the significance of their presence in these biofuels should not be overstated [17,18].

While extractions of fire debris samples at this higher temperature are less common, they can provide additional markers to identify a biodiesel blend once one has been identified, and therefore should be considered when analyzing samples suspected to contain biodiesel.
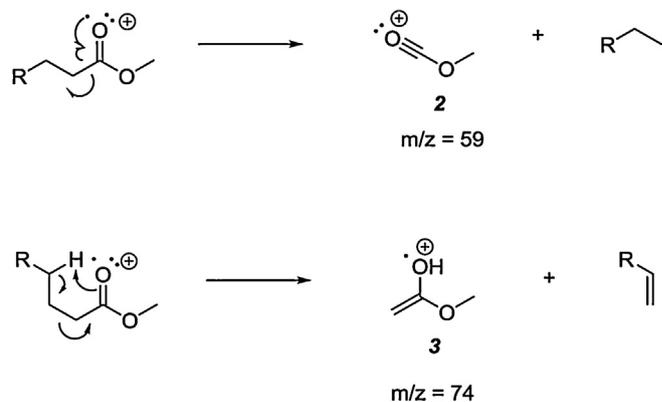
### 3.3. Extracted ion chromatograms

The analysis of petroleum products is helped greatly by extracting ions of interest from the chromatograms. This allows the analyst to find diagnostic patterns of different distillates that may be hidden by mixtures or the sample matrix. Table 2 lists the common ions extracted and the class of hydrocarbons to which those ions correlate, and includes the ions that are useful in identifying methyl esters.

Saturated, long-chain FAMEs have mass spectra that are very similar to alkanes, but methyl esters have their own identifying fragments that will prove to be useful in identifying FAMEs in fire debris samples, as shown in Scheme 1. The acylium ion **2** that results from α-cleavage of the C–C ester bond has $m/z = 59$. In addition, McLafferty rearrangement gives a radical cation **3** with $m/z = 74$. These two peaks in a chromatogram are therefore indicative of a methyl ester. The difference can be seen by comparing the extracted ion chromatograms of B5 and B20 biodiesel; this is done in Figs. 6 and 7.

**Table 2**
Common ions extracted to identify petroleum distillates, and those useful for identifying FAMEs.

| Hydrocarbon class | Ions of interest |
|---|---|
| Alkanes | 43, 57, 71, 85 |
| Cycloalkanes and alkenes | 55, 69 |
| n-alkylcyclohexanes | 82, 83 |
| Alkylbenzenes | 91, 106, 120, 134 |
| Indanes | 117, 118, 132 |
| Naphthalene | 128 |
| Alkylanthracenes | 178, 192, 206 |
| **Fatty acid methyl esters** | **59, 74** |

The extracted ion chromatograms for diesel and B20 biodiesel using $m/z = 57$ for alkanes show little difference between the two. Neglecting to extract the $m/z = 59$ and 74 ions may therefore lead to the misidentification of a biodiesel blend as a petroleum distillate if the FAME peaks cannot be clearly seen in the total ion chromatogram. In contrast to the above chromatograms, when the $m/z = 74$ ions are extracted, the difference between diesel fuel and biodiesel is much more obvious. The $m/z = 59$ ions can also be extracted to help identify the FAME components, but the $m/z = 74$ ion is more abundant, making it more useful in identifying methyl esters. Making use of these extracted ions, it is possible to detect the FAMEs of the biodiesel in various blends even when their presence is obscured in the total ion chromatogram. The use of these ions should therefore be considered any time the presence of a biodiesel product is suspected.

### 3.4. Microbial degradation

The effects of microbial degradation on gasoline when it is exposed to soil have been well-studied [19], and this examination has more recently been broadened to other petroleum-based ignitable liquids [20]. Previous studies have examined the biodegradation of biofuels in natural environments [21]; many of these studies used commercially available bacteria [22] or allowed the fuels to degrade in the presence of seawater [23] or rainwater [24], in order to speed the process. Indeed, many of these studies show >90% of degradation of FAMEs within one month under their respective conditions. The literature of biodegradation reveals a significant effect of the environment on degradation. In marine water [23,25] and rainwater [24], FAMEs and n-alkanes were found to degrade at similar rates, and no difference was observed between the degradation of saturated and unsaturated FAMEs. Under controlled laboratory conditions of biofuels mixed with diesel and gasoline exposed to mineral nutrients, the presence of biodiesel increased the rate of degradation of diesel and gasoline as measured by $CO_2$ evolution [26]. In sand, the presence of biodiesel appeared to have no effect on the degradation of hydrocarbons [27]. In general, samples exposed to soil have shown similar results, specifically that biodiesel is degraded more easily than hydrocarbons, and unsaturated FAMEs are degraded more quickly than saturated FAMEs [28]. The higher rate of degradation of unsaturated FAMEs is expected, as the higher reactivity of alkenes relative to alkanes makes them more susceptible to oxidative metabolism [14b]. Because the biodiesel blends contain FAMEs of vegetable oil, we hypothesized that microbial degradation would be a significant factor in the analysis of biodiesel.
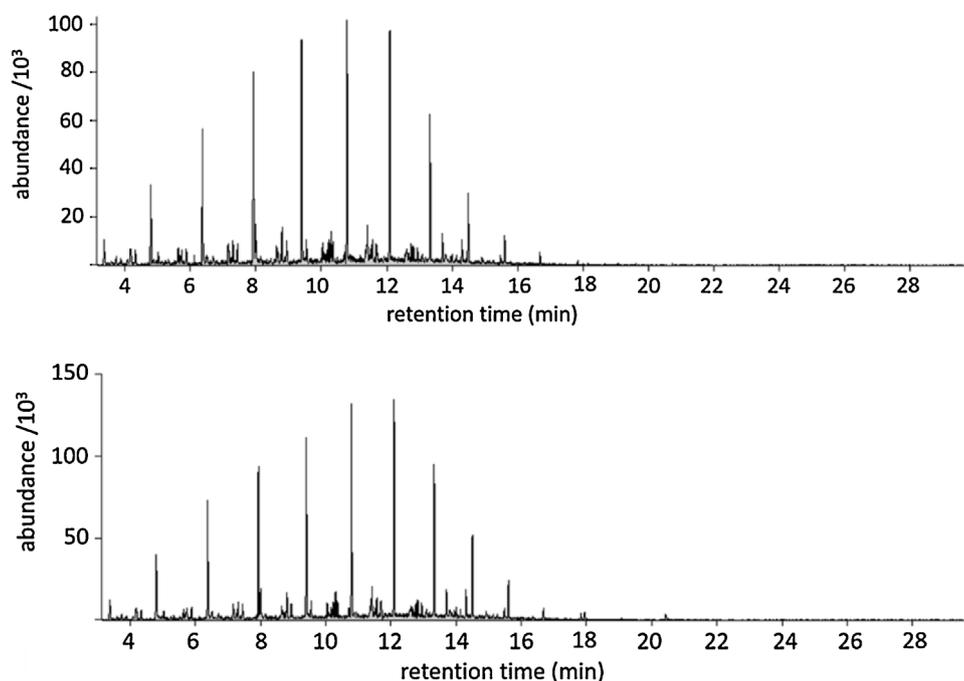
**Fig. 6.** Extraction ion chromatogram ($m/z$ = 57) for a heated-headspace (75 °C) sample of B5 (top) and B20 (bottom), showing their similar $n$-alkane profiles.

The chromatograms that resulted from exposing B20 biodiesel to soil samples taken from the campus of Pacific Lutheran University over 2, 7, 14, 30, and 60 days, after headspace analysis at 75 °C, are shown in Fig. 8. Similar chromatograms for the other fuels are provided in the Supplemental Information. Immediately apparent is that the peaks corresponding to FAMEs decrease significantly after two days. An expanded view of the FAME peaks of the extracted ($m/z$ = 74) ion chromatograms is shown in Fig. 9, and shows that the FAME peaks are virtually undetectable after 14 days. The relative

amounts of FAMEs in each sample can be seen semi-quantitatively by comparing their abundances to that of the 1-chlorohexadecane internal standard. Fig. 10 shows the dramatic decrease in abundance of the FAME peaks, becoming unobservable between 14 and 30 days, while the relative abundances of the $n$-alkanes remain constant.

As can be seen in the Supplementary Information, exposing the homemade B100 fuel to soil resulted in the presence of the same oxygenated small molecules (primarily hexanal) as heating the fuel to 100 °C. This was the case even in samples in soil analyzed at



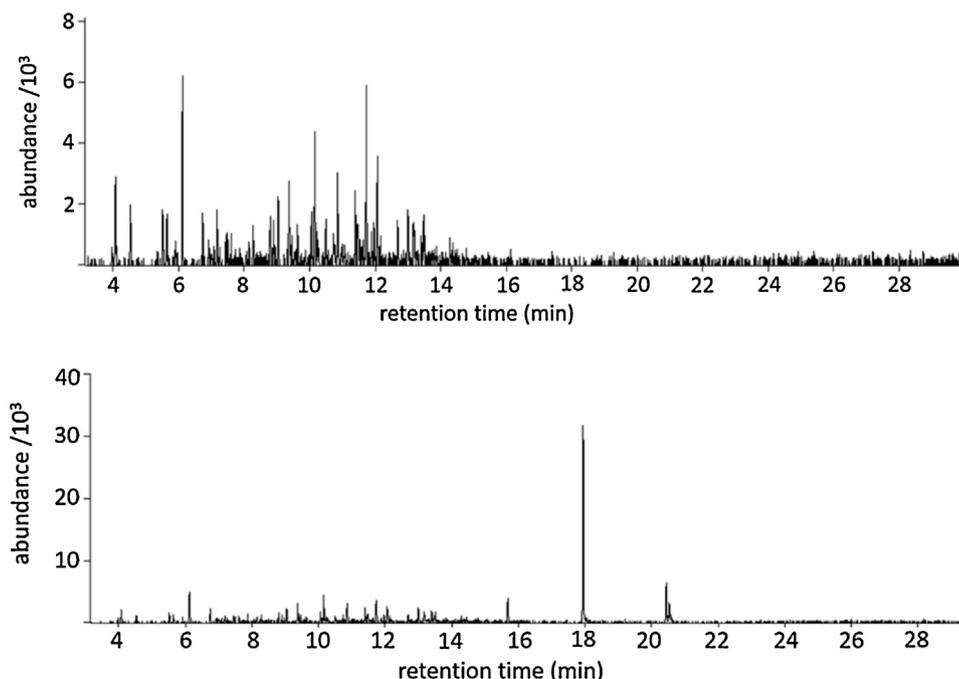**Fig. 7.** Extracted ion chromatograms ($m/z$ = 74) for a heated-headspace (75 °C) sample of B5 (top) and B20 (bottom). The FAME peaks can clearly be seen in the B20 profile.
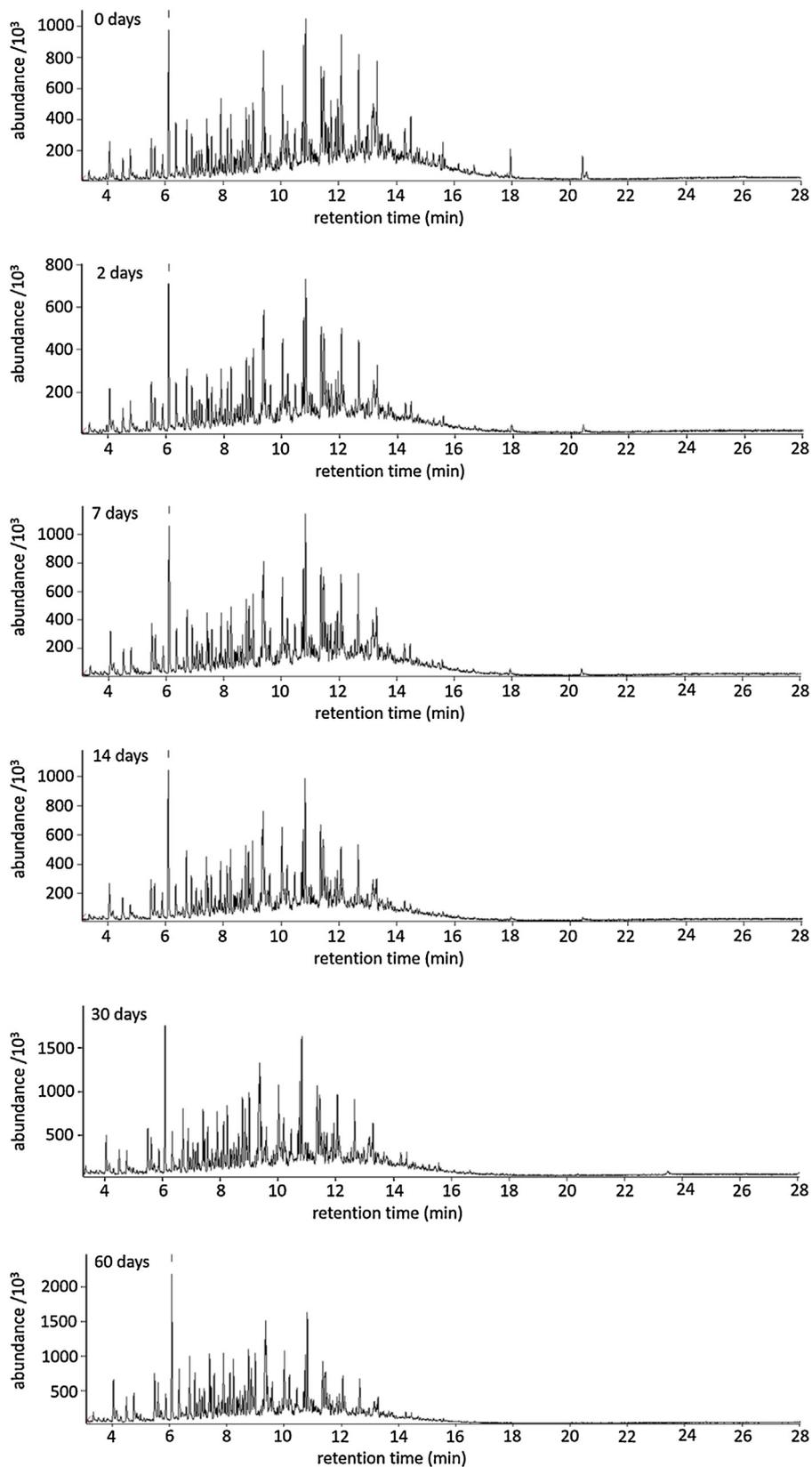
**Fig. 8.** Total ion chromatograms of B20 biodiesel after exposure to soil over a period of 60 days. Heated-headspace analysis was performed at 75 °C.

75 °C. This suggests that the oxidative decomposition of B100 can occur metabolically [15] as well as thermally. A similar result was observed with the commercially-obtained B99 blend, but not with the other, lower-FAME containing blends.

Many previous studies of the degradation of FAMEs have followed the metabolism of the esters by measuring the amount of carbon dioxide released by the sample. While we did not directly measure $CO_2$ production from the biodiesel samples, the observation of the
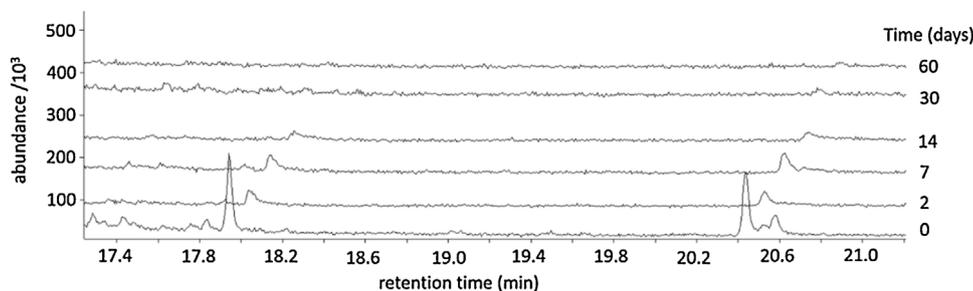
**Fig. 9.** Extracted ($m/z$ = 74) ion chromatograms of B20 over 60 days.

release of gas from the cans when they were opened after cooling suggested that the respiration process was taking place. We therefore examined the total ion chromatograms to see if any products of degradation could be observed. Aside from the disappearance of the FAME peaks over time, however, the rest of the total ion chromatograms remained essentially identical over 60 days. We conclude that the respiration of the FAMEs resulted in their conversion to $CO_2$ and small, volatile hydrocarbons that escaped from the cans and were therefore not observed in the heated headspace samples.

For comparison, we looked at the behavior of some of the alkane and aromatic peaks relative to the internal standard. Specifically, we examined the peaks corresponding to undecane, dodecane, pentadecane, and hexadecane, as well as 1,2,4-trimethylbenzene, methylindan 1-methyl-2,3-dihydro-1H-indene), and methyltetralin (1,2,3,4-tetrahydro-1-methylnaphthalene). These components were chosen because their abundances are relatively high in the samples before exposure to soil, so any degradation would be easier to detect. The results, shown in Fig. 11, reveal that the alkanes and aromatic compounds are not significantly affected by the presence of the soil. The abundances of the alkanes are especially consistent over the course of 60 days in the soil. The aromatic compounds, while more variable, do not change in a consistent way during the experiment.

The finding that the abundances of $n$-alkanes and aromatic compounds do not significantly change during the exposure to soil is surprising, given recent studies which show that these compounds can degrade within a few days upon weathering [19–28]. We recognize that there are several factors that can affect

the activity of microbes in the soil; among these are humidity, light, and heat. The conditions under which the fuels were exposed to soil in the present study almost certainly affected the microbial activity, and therefore the behavior of the $n$-alkanes and aromatics. The recent literature, which shows a wide variation on the behavior of FAMEs compared to hydrocarbons when exposed to different conditions (see above), leads us to devise two general hypotheses. The first is that the presence of FAMEs under the conditions described herein made the microbial degradation of hydrocarbons less favorable. The relative consistency of the abundances of the $n$-alkanes and aromatics in B5 when exposed
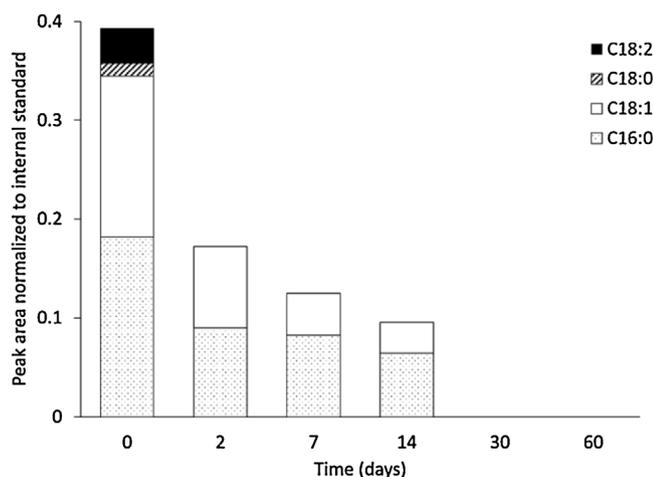




**Fig. 10.** Abundances of FAME components of B20 biodiesel relative to 1-chlorohexadecane upon exposure to soil over a period of 60 days. The sample was analyzed by heated-headspace at 75 °C.

**Fig. 11.** Abundances of selected $n$-alkane (top) and aromatic (bottom) components of B20 biodiesel relative to 1-chlorohexadecane upon exposure to soil over a period of 60 days. The sample was analyzed by heated-headspace at 75 °C.
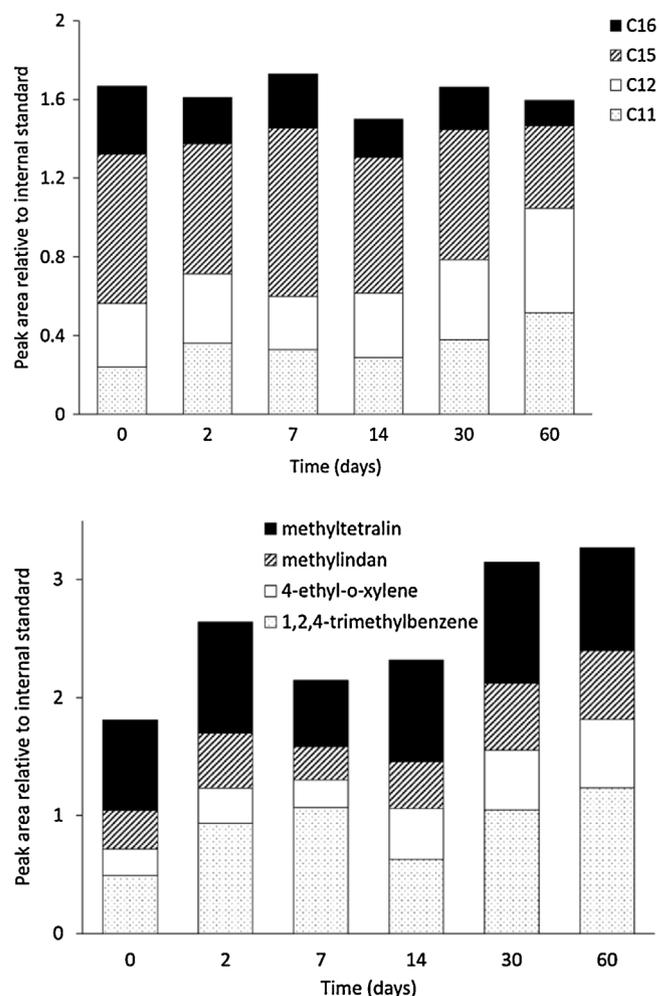
to soil (Supplementary Information) seems to disprove this hypothesis—it would be expected that decreasing the fraction of FAMEs in a fuel sample would lead to observable degradation of the hydrocarbons, and this was not the case. The other hypothesis is that something about the conditions used herein affected the microbial activity toward the hydrocarbon components of each fuel. The soil samples used in the present study were obtained after several dry days, so the humidity of the samples was likely quite low. The potting soil was used "as-is", with no manipulation before

exposure to the fuels. Samples were kept under dark and dry conditions throughout the 60-day experiments, in order to maintain uniform conditions as much as possible. Because there are so many variables to consider, each of which can significantly affect the activity of the soil toward degradation of the biofuels, a more comprehensive study of the weathering of biodiesel when these conditions are changed is certainly warranted. The results presented herein should be taken as preliminary results, and a starting point for future work.
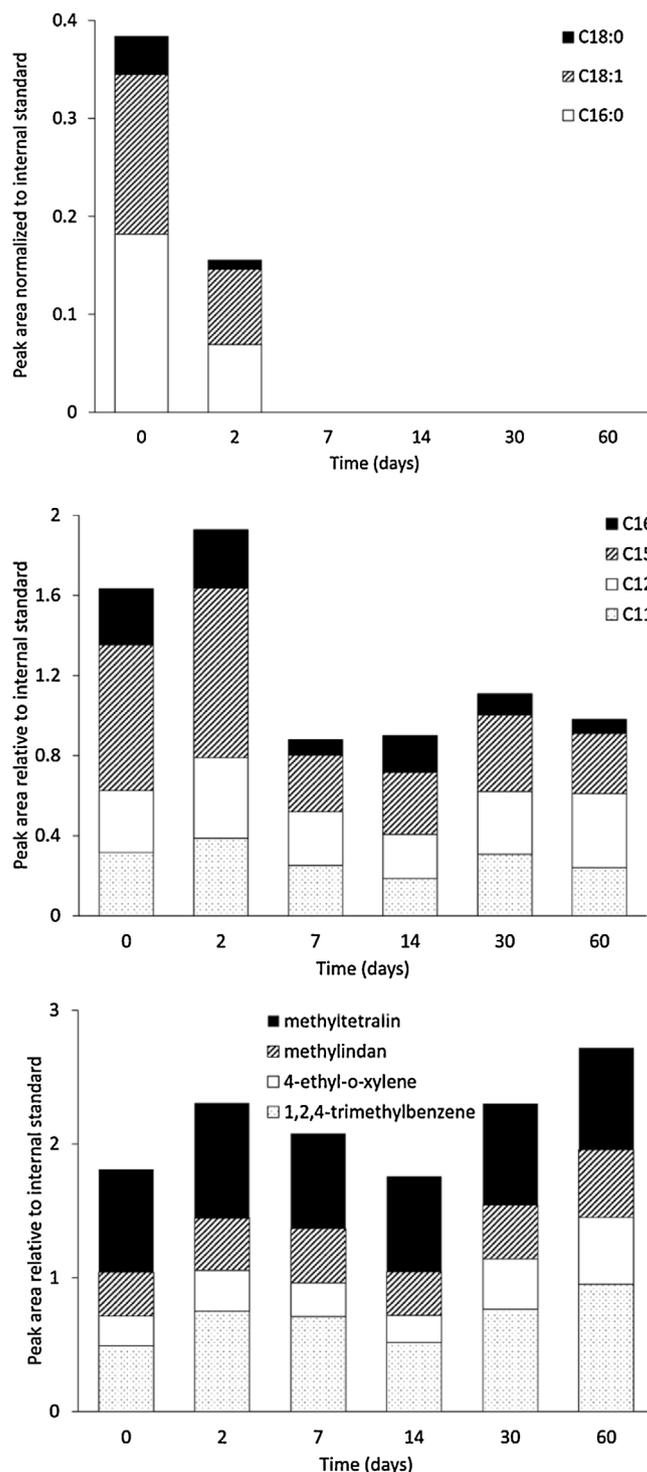


**Fig. 12.** Abundances of FAME components (top), selected *n*-alkane (center), and aromatic (bottom) components of B20 biodiesel relative to 1-chlorohexadecane upon exposure to potting soil over a period of 60 days. The sample was analyzed by heated-headspace at 75 °C.

We hypothesized that the degradation of FAMEs in biodiesel would be accelerated in commercially available potting soil, which should contain a higher number of microbes. In fact, we observed that the peaks corresponding to FAMEs in B20 biodiesel disappeared after seven days in the potting soil (Fig. 12). In the B50 and B99 samples, the FAME peaks are observable, but dramatically diminished compared to the control samples (Supplementary Information). We conclude that microbial degradation of FAMEs present in biodiesel is a significant factor in the analysis of these fuels. Comparisons of the FAME abundances to the internal standard show that the FAMEs are unobservable only after 7 days. The degradation of the biodiesel components is clearly much faster in potting soil. The abundances of the aromatic compounds remain roughly the same throughout the 60 days, much like the samples observed in regular soil. The heavier alkanes, however, appear to decrease slightly after 2 days, as can be seen from the $C_{15}$ and $C_{16}$ abundances in Fig. 12. The lighter alkanes, as represented by $C_{11}$ and $C_{12}$, do not change significantly over the course of 60 days. The difference between this result and the consistency of the abundances of the alkanes in regular soil is likely due to the increased number (and possibly different types) of microbes in the potting soil. For example, once the FAMEs are consumed, the higher alkanes may become more susceptible to microbial metabolism. As usual, however, these results must be interpreted with care, and more studies with different types of soil (preferably from different locations, and in different seasons) should be carried out to further expand the database of microbially degraded fuels.

Because the FAME components of the biodiesel blends disappeared so quickly, and to see if the presence of FAME components in the blends can be seen at higher analysis temperatures, we repeated the microbial degradation experiment and analyzed the samples at 100 °C instead of 75 °C. We hypothesized that the higher analysis temperature would reveal FAME components in the biodiesel blends after longer periods of time. In regular soil, this was true (Fig. 13); the C16:0 FAME was observable throughout the 60 days, although its abundance greatly decreased (along with the abundances of the other FAMEs) after 2 days. The trends observed with the alkanes and aromatic compounds were similar to those seen for the samples analyzed at 75 °C.

The behavior of B20 in potting soil analyzed at 100 °C is similar to that analyzed at 75 °C, as shown in Fig. 14. The FAME peaks are observed for a longer period of time (each of them can be seen after 14 days, but then disappear after 30 days), and the peaks corresponding to heavier alkanes diminish over time. The peaks corresponding to the aromatic compounds followed here seem to stay consistent before decreasing slightly after 60 days.

It is interesting to examine the rates at which the FAMEs appear to be consumed at each analysis temperature. Comparing the relative areas of the peaks vs. the internal standard, plots of the abundances of C16:0, C18:1, and C18:0 are shown in Fig. 15. When analyzed at 75 °C, the peaks corresponding to C16:0 and C18:1 diminish at approximately the same rate over 2 days, after which C18:1 is consumed more quickly than C16:0. Because the amount of C18:0 is so small relative to the others, and disappears completely after two days, its rate of disappearance is difficult to interpret at this analysis temperature. At 100 °C, the rates of consumption of C16:0 and C18:1 appears to be similar throughout the 60 days of analysis, while the disappearance of the C18:0 peak is a little slower. The similarity in rate of consumption of C16:0 and C18:1 is unexpected, since many previous reports have concluded that unsaturated fatty acids and FAMEs are degraded more quickly by microbes than saturated ones by oxidative metabolism [15,28]. However, hydrolysis of a saturated FAME to its fatty acid could allow for β-oxidation, and another pathway for degradation [14b,29]. The fatty acid products of hydrolysis and β-oxidation would not be observed in the TIC (see above), so this pathway is
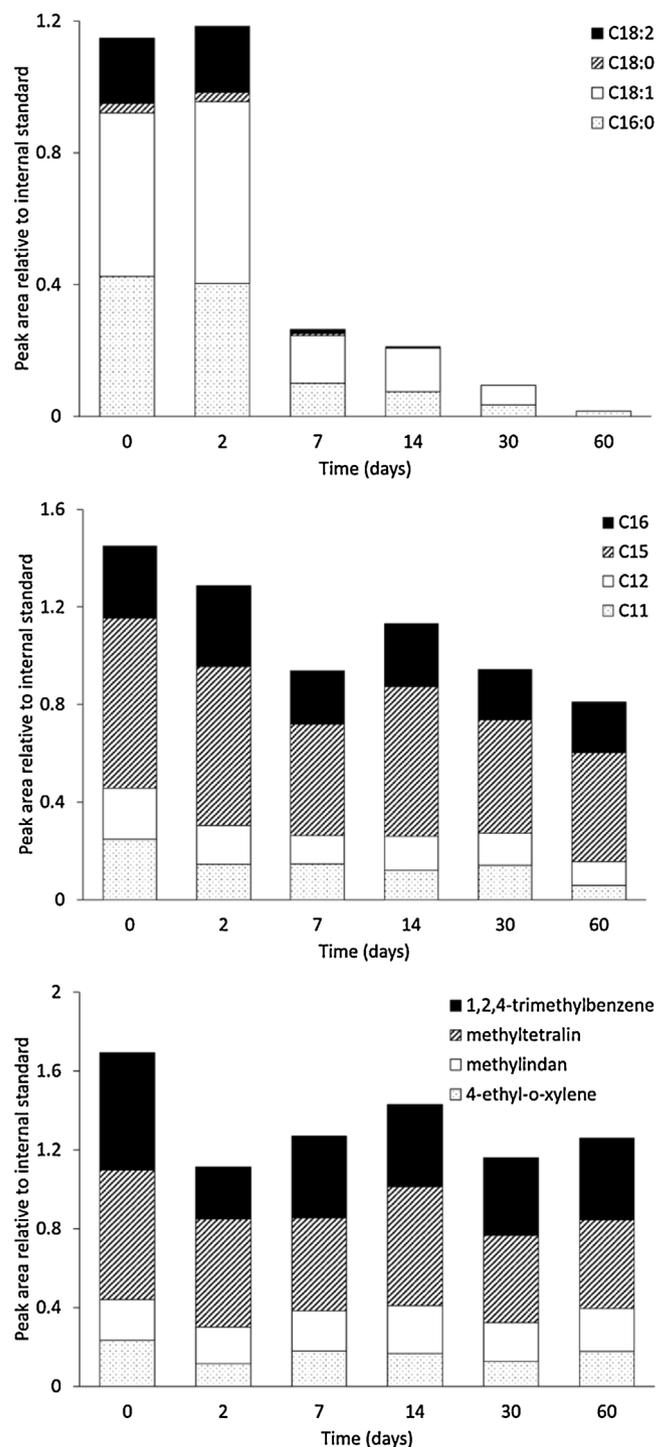


Fig. 13. Abundances of FAME components (top), selected *n*-alkane (center), and aromatic (bottom) components of B20 biodiesel relative to 1-chlorohexadecane upon exposure to soil over a period of 60 days. The sample was analyzed by heated-headspace at 100 °C.

only a proposed one at this stage, but one that can explain the similarities in the degradation rates of saturated and unsaturated FAMEs. The fact that the general presence of biodiesel components is greatly diminished after 7 days, even when analyzed at 100 °C, is significant because it illustrates how quickly biodiesel blends can be degraded to appear very similar to diesel.

The behavior of the thermal decomposition products of B100 are shown in Fig. 16 (after exposure to soil) and Fig. 17 (after
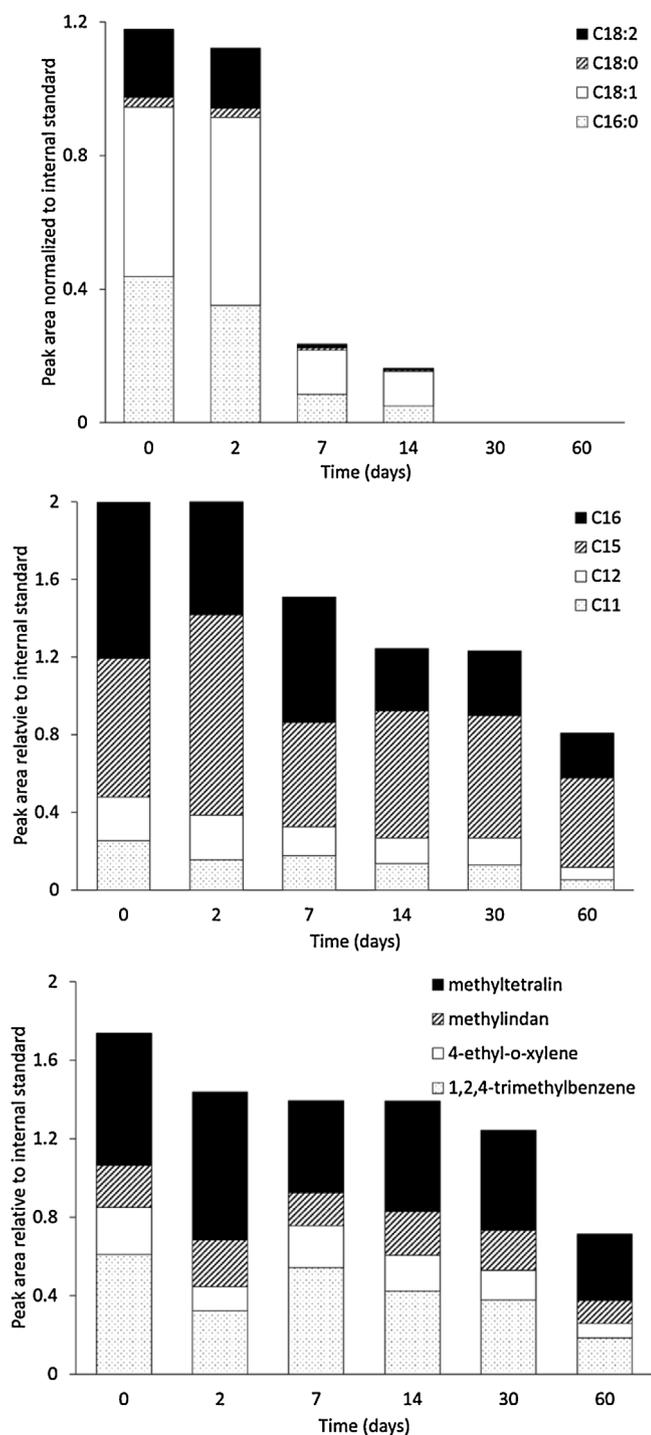
**Fig. 14.** Abundances of FAME components (top), selected *n*-alkane (center), and aromatic (bottom) components of B20 biodiesel relative to 1-chlorohexadecane upon exposure to potting soil over a period of 60 days. The sample was analyzed by heated-headspace at 100 °C.



**Fig. 15.** Abundances of C16:0, C18:1, and C18:0 in B20 biodiesel exposed to soil over 60 days, analyzed at 75 °C (top) and 100 °C (bottom). The abundances are shown relative to 1-chlorohexadecane as an internal standard.

time were constant throughout this study, small changes may have had a large effect on the amounts observed; therefore, care must be taken when interpreting these results. A more detailed study of these factors involved in the thermal and oxidative degradation of biodiesel is warranted, and will be the focus of future work.

### 3.5. Evaporation studies

Weathering of samples is also known to significantly change the chromatograms of petroleum products, generally through the disappearance (through evaporation) of the lighter hydrocarbons relative to the heavier components [30]. Since the FAMEs present in biodiesel are relatively non-volatile and appear at higher retention times, we predicted that subjecting samples to evaporation would not affect the FAME peaks to the same extent as the diesel peaks. To a large extent, we found this to be true. For all of the fuels subjected to evaporation, the lighter, lower-retention time hydrocarbon components disappeared first, giving chromatograms that favored the heavier hydrocarbons and the FAMEs, Fig. 18. Similar chromatograms for the other fuels can be found in the Supplemental Information. The diesel (hydrocarbon) part of the chromatograms shows the expected disappearance of the lighter alkanes through evaporation, with a corresponding increase in the relative abundance of the heavier alkanes. It is not

exposure to potting soil). The abundances of each of the compounds in the B100 sample are variable up to seven days. After that time, they all appear to decrease uniformly. Assuming that these compounds are formed during the heating of the samples in the oven, the amounts of each present in a given sample is sensitive to the heating time and temperature, as well as the amount of FAMEs present (as sources of these compounds). While every effort was made to ensure that the analysis temperature and
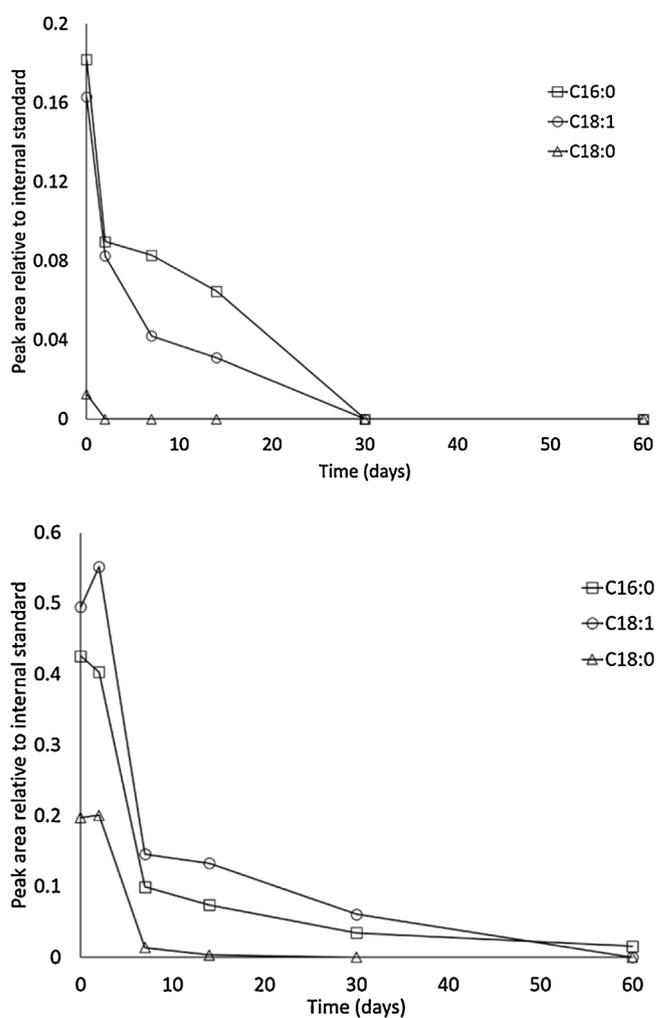
**Fig. 16.** Abundances of FAME components (top) and oxygenated decomposition products (bottom) of homemade B100 biodiesel relative to 1-chlorohexadecane upon exposure to soil over a period of 60 days. The sample was analyzed by heated-headspace at 100 °C.
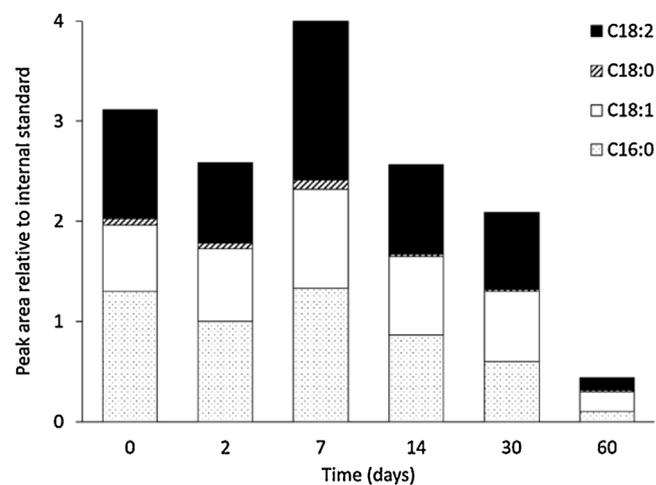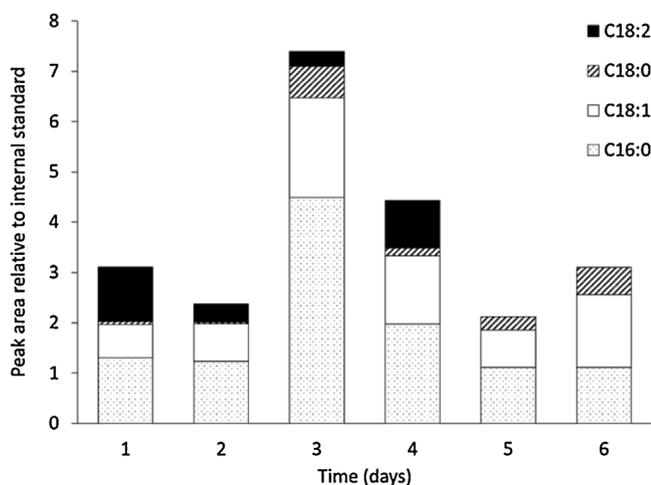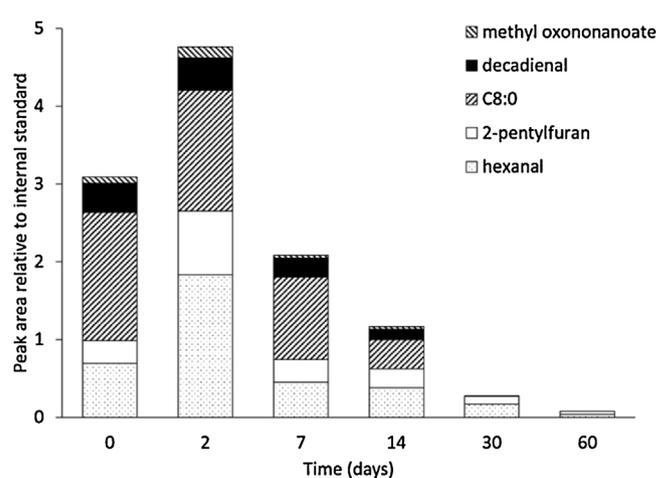
**Fig. 17.** Abundances of FAME components (top) and oxygenated decomposition products (bottom) of homemade B100 biodiesel relative to 1-chlorohexadecane upon exposure to potting soil over a period of 60 days. The sample was analyzed by heated-headspace at 100 °C.

until the sample is 75% evaporated that a decrease in the abundance of the FAME peaks is observed, when the abundance of the di-unsaturated methyl linoleate (C18:2) decreases relative to the saturated and monounsaturated FAMEs. It should be noted that temperatures of up to 250 °C were required to achieve 75% evaporation of the B20 sample (as well as the other samples). Therefore, simple evaporation by exposure would not result in the chromatograms shown here. However, samples exposed to fire (where temperatures can easily exceed 1000 °C) may exhibit chromatograms similar to those observed here. As evaporation continued, the presence of the FAMEs in each fuel became more prominent. In fact, after the B5 fuel had evaporated by 50%, the FAME peaks could begin to be observed in the extracted ion chromatogram (Fig. 19). As expected, the evaporation rates of the fuels depended on the fraction of biodiesel present. The B5 fuel evaporated readily by 25% without the aid of heat, while the B99 and B100 fuels did not evaporate at all without significant heating (>250 °C); even the B20 fuel needed significant heating to evaporate more than 75%.

In the Supplemental Information, it can be seen that heating the B99 and B100 biodiesel samples did not result in the observation of the same decomposition products that were seen in the 100 °C heated-headspace samples of the B100 fuel. This somewhat surprising result can be explained by noting that in the sealed can, the products were adsorbed onto the activated charcoal strip, while in an open beaker the products could be formed, and then evaporate along with the hydrocarbons of the biodiesel fuel. The highest-boiling compound in Table 1, methyl 9-oxononanoate, boils at 250 °C. Therefore, we propose that the same decomposition products are formed, and then evaporate away.

### 3.6. Matrix studies

It is well known that the composition of the matrix in which fire debris is contained has a significant effect on the analysis and identification of ignitable liquid residues. This is especially true of the identification of petroleum distillates, since petroleum-based products are found in so many household products [18]. With this in mind, we performed control analyses of commonly-encountered substrates to see if any FAMEs could be identified in the absence of any biodiesel. Therefore, samples of carpet, cedar lumber, a pine tree branch, and samples of cotton and polyester clothing, both unburned and burned in the absence of any fuel, to see if any petroleum or FAME compounds would be observed. The data, shown in the Supplemental Information, reveal that under the analysis conditions used herein, none of the substrates analyzed showed any peaks that could be mistaken for FAMEs. More studies remain to be done on a number of different substrates.
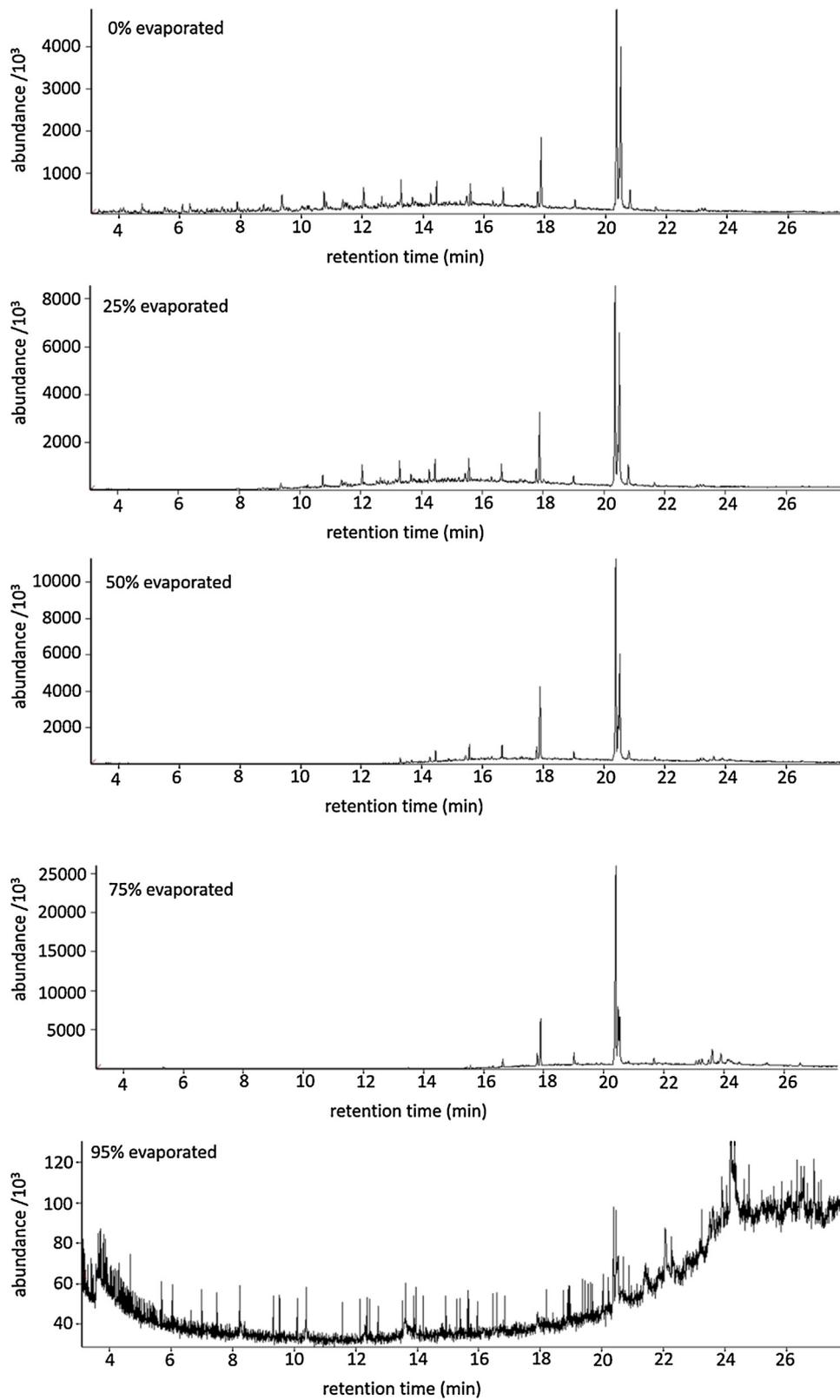
**Fig. 18.** Total ion chromatograms for the evaporation of B20 biodiesel, from top to bottom: neat sample, 25% evaporated, 50% evaporated, 75% evaporated, and 95% evaporated.
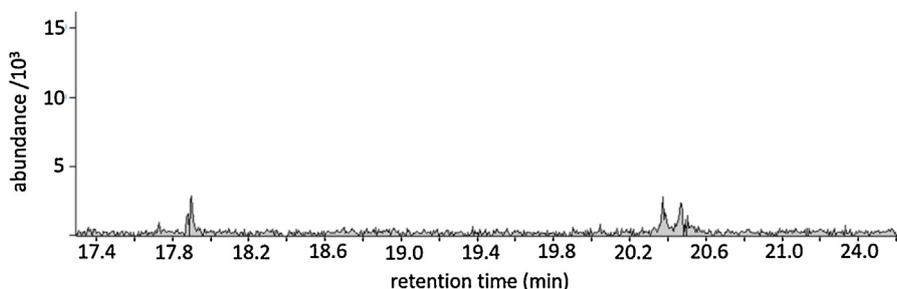
**Fig. 19.** Extraction ion (*m/z* = 74) chromatogram of B5 biodiesel after 50% had evaporated. Peaks corresponding to FAME components can begin to be observed at this point, but not before, in the case of the B5 blend.

## 4. Conclusions

The behavior of different biodiesel blends under conditions typically found at fire scenes has been investigated, showing that weathering, microbial degradation, and matrix effects can all contribute to complications in the analysis of these increasingly common fuels. Specifically, we have found:

- Samples analyzed by heated headspace underrepresent the presence of biodiesel in the blended samples, to the extent that at lower fractions of biodiesel, at first glance a sample looks very similar to a pure heavy petroleum distillate.
- The extraction temperature has a significant effect on the chromatogram. At higher extraction temperatures, peaks corresponding to FAMEs are more prominent. Higher temperatures can therefore be helpful in identifying a biodiesel product.
- The use of extracted ions *m/z* 59 and 74 for the presence of FAMEs is a powerful way to determine whether a biodiesel product may be present in a sample.
- Biodiesel blends are subject to microbial degradation, losing their FAME peaks very quickly and making weathered biodiesel samples appear very similar to diesel fuel.
- The FAMEs present in biodiesel blends are relatively resistant to evaporation under ambient conditions, making their presence more obvious in an evaporated biofuel sample.
- A number of matrices studied herein, commonly found at fire scenes, do not appear to contain significant FAME components that could interfere with analysis. Studies with a wider variety of matrices are needed.

The number of fire debris samples submitted for analysis that contain some kind of biodiesel is likely to increase significantly over time. The analytical data obtained herein should prove to be useful for the fire debris analyst when interpreting the data obtained from these samples.

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.forsciint.2016.03.040.

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