TECHNICAL NOTE

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Alternative Fuels in Fire Debris Analysis: Biodiesel Basics

ABSTRACT: Alternative fuels are becoming more prominent on the market today and, soon, fire debris analysts will start seeing them in liquid samples or in fire debris samples. Biodiesel fuel is one of the most common alternative fuels and is now readily available in many parts of the United States and around the world. This article introduces biodiesel to fire debris analysts. Biodiesel fuel is manufactured from vegetable oils and/or animal oils/fats. It is composed of fatty acid methyl esters (FAMEs) and is sold pure or as a blend with diesel fuel. When present in fire debris samples, it is recommended to extract the debris using passive headspace concentration on activated charcoal, possibly followed by a solvent extraction. The gas chromatographic analysis of the extract is first carried out with the same program as for regular ignitable liquid residues, and second with a program adapted to the analysis of FAMEs.

KEYWORDS: forensic science, biofuel, vegetable oil, animal fat, FAME, fatty acids, diesel fuel, gas chromatography

Alternative fuels are materials other than petroleum-derived products used to power an engine. Although alternative fuels have existed for many years, their use has been so far somewhat marginal. However, this is about to change. The present situation of petroleum market instability, limited availability of crude oil, refineries reaching their peak production, and, last but certainly not least, the serious impact of the use of petroleum-based fuels on the environment, have all positively impacted the spread of alternative fuels. In the United States, the popularity of sport utility vehicles and large pick-up trucks with low fuel efficiency is decreasing. Manufacturers have responded first by marketing smaller cars with more fuel-efficient engines that have been in use for many decades in Europe and other countries around the world. More recently, some manufacturers such as Honda, Toyota, Ford, and Chevrolet have proposed hybrid vehicles; these vehicles consist of an electrical engine combined with an internal combustion engine and present an impressively low petroleum fuel consumption (up to *c.* 60 miles/gallon or down to *c.* 4 L/100 km) (1). This solution, while interesting in the short term, still uses petroleum-based fuels and may not be sustainable in the long term. In the last few months, and particularly in the United States, the number of reports from the media on the use of biodiesel and gasolines E10 and E85 has dramatically increased. The National Biodiesel Board (NBB), the U.S. national trade association representing the biodiesel industry for research and development, reported that the production of biodiesel in the United States increased from 0.5 million gallons (1.9 million liters) in 1999 to 75 million gallons (284 million liters) in 2005 (2). Many U.S. governmental agencies have also started to switch their vehicle fleets to biodiesel and other alternative fuels. For example, in October 2005, the City of Maryville (Tennessee) announced that it would operate all its diesel fuel vehicles with biodiesel (3). Also, some U.S. military bases have been using biodiesel for a few years. Last year, the U.S. military used *c.* 6 million gallons (23 million liters) of biodiesel, quite a significant quantity, although still quite a small portion of the several billion gallons of diesel fuel used every year (4). Today, there are 65 commercial biodiesel production plants in the United States with an annual production capacity of 395 million gallons (1.5 billion liters) and 50 more commercial production facilities are under construction (5,6). According to the NBB, there are also more than 1,500 retailers in the United States from which biodiesel can be purchased (7).

When fire debris analysts are trained, they are taught that most ignitable liquid residues (ILR) they will encounter during their careers are petroleum-based liquids. This is true and will likely remain true for many years to come. A few liquids, mostly oxygenated compounds and wood distillates (terpenes), are not petroleum-based and are encountered on a sporadic basis. One caveat of this phenomenon is that fire debris analysts usually do not gain an extensive experience with nonpetroleum-based liquids. With the rapid emergence of alternative fuels, it is likely that unknown liquids (or fire debris) containing such fuels will be more often submitted to the crime laboratory and examined by the fire debris analyst. While many criminalists are aware of the existence of alternative fuels such as biodiesel, there is a lack of knowledge regarding their composition and the characteristics they exhibit when analyzed. The purpose of this paper is to introduce fire debris analysts to biodiesel fuels. General information about the different types of biodiesel fuels, their characteristics, and their production is provided. This is followed by a brief description of the forensic approach to take with samples suspected of containing biodiesel residues.

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Biodiesel

History

In 1893, German inventor Rudolf Christian Carl Diesel obtained the patent for his internal combustion engine, now known as the diesel engine (8). Rudolf Diesel first tried to run his engine with small, pulverized carbon particles. However, this did not work well and he used different petroleum-based fuels, and more particularly kerosene, to pursue the development of his engine. However, in 1900, when he introduced his internal combustion engine at the Exposition Universelle (World Exhibition) in Paris, France, he used peanut oil as fuel (9). Diesel was able to demonstrate that his engine ran perfectly well on vegetable oils. The idea behind that was to allow farmers to produce their own fuels to run their "diesel" engines. So, contrary to popular belief, the "diesel" engine was not developed to be fueled exclusively with diesel fuel and was actually fed with vegetable oils for many years. As a matter of fact, in 1912 Rudolf Diesel stated that (9): "the use of vegetable oils for engine fuels may seem insignificant today. But such oils may become in the course of time as important as petroleum and the coal tar products of the present time." The copious availability of crude oil and the emergence of its refinement led to the wide production of diesel fuel and its accepted "standard" use in diesel engines. From the 1920s until today, this was a much more economical solution to fuel diesel engines.

Definition

Biodiesel is part of the family of biofuels and is a term used to define a fuel used in lieu of diesel fuel and that is produced from a biological source: vegetable oils and/or animal oils/fats (10). When produced from vegetable oils, the terms vegidiesel or vegifuel are also used, but to a lesser extent. Biodiesel can be used pure or blended with regular diesel fuel. ASTM International defines a biodiesel blend as (11): "a blend of biodiesel fuel with petroleum-based diesel fuel." Biodiesel blends are often designated with the abbreviation BXX, where XX represents the volume (in percent) of biodiesel fuel in the blend (11). Thus, a blend of 80% diesel fuel and 20% biodiesel is designated as B20. A fuel consisting of pure biodiesel is designated as B100.

Specifications

Although biodiesel is of different chemical composition than diesel fuel, it exhibits some similar properties. Table 1 shows a comparison of several properties of pure biodiesel (B100), a biodiesel blend (B20), and pure diesel fuel (11–14). It is important to understand that these data are approximate in nature and variations between different sources of fuel may occur. Some of these values are also minima set by standards, such as the ASTM standards D6751-03a for biodiesel fuels and D975-05 for diesel fuels (11,12).

Some values such as the cetane number are similar in nature due to the fact that both liquids are used for the same function. From a fire investigation perspective, one of the most significant differences between biodiesel and diesel fuel concerns the flash point. While both diesel fuel and biodiesel are combustible liquids (flash points above 37.8°C or 100°F as defined by the National Fire Protection Association (15)), the flash point of pure biodiesel is much higher (at least 130°C or 266°F). Thus, it does not make a good accelerant because it is difficult to ignite. Under these circumstances, fire debris analysts will rarely encounter fire debris in which biodiesel residues are present as accelerants. More reasonably, unburned samples soaked in biodiesel might be collected by fire investigators at the scene of an attempted arson, as it has been the case many times in the past with samples soaked with engine oil or vegetable oil. Alternatively, fire debris analysts may encounter debris in which liquid samples of biodiesel are present or more simply pure (unknown) liquids actually constituted of biodiesel.

From a boiling point perspective, diesel fuel has an initial boiling point around 185°C (365°F) and an end point around 345°C (653°F). Average initial boiling point and end point of biodiesel produced with soybean oil have been reported to be 299°C (570°F) and 346°C (655°F), respectively (16). Lower initial boiling points have also been observed with other oils, such as tallow oil; however, they typically remain above 200°C (392°F) (16). This means that biodiesel is less subject to evaporation than diesel fuel and will likely remain adsorbed on debris for a very long period of time.

Liquids with higher boiling points usually exhibit higher melting points also. The melting point of biodiesel is one of its main disadvantages from a functional point of view. Usually, diesel fuel solidifies starting around −18°C (0°F), while biodiesel solidifies around 4°C (39°F). The higher melting point of biodiesel creates issues regarding its use in wintertime. This is a good property to remember, as it may serve as a very quick preliminary test to differentiate between biodiesel and diesel fuel.

Biodiesel and diesel fuel are completely miscible and mixtures of B5, B20, and B50 are most often encountered. Other ratios are available, but less common. Properties of each biodiesel blend present variations between the two extreme values exhibited by pure biodiesel and pure diesel fuel.

Advantages

The main advantages of biodiesel are that it is a renewable fuel, it reduces the dependence on fossil fuel, and it reduces the emission of air pollutants (10). Additionally, it is relatively easy to manufacture and is a great way of recycling waste frying oils (WFO) or waste vegetable oils (WVO). The reduction in air

TABLE 1—*Comparison of some properties of biodiesel (B100), a biodiesel blend (B20), and diesel fuel (11–14).*

Fuel	Biodiesel B100	Biodiesel B20	Diesel Fuel
Boiling point	> 200°C (392°F)	min 171°C (340°F)	∼ 160°C (320°F)
Melting point	∼ 4°C (39°F)	—	∼ −25°C (−13°F)
Flash point (closed cup)	min 130°C (266°F)	66°C (150°F)	min 52°C (126°F)
Specific gravity (water = 1)	0.88	0.88	0.85
Vapor pressure (mmHg)	< 2	<1	0.40
Cetane number	min 47	—	min 40
Autoigniton temperature	—	610°C (1131°F)	316°C (601°F)

pollutants compared with diesel fuel is far from negligible. According to the EPA study on the exhaust emissions of B100 compared with those of diesel fuel, total unburned hydrocarbons are reduced by 67%, carbon monoxide by 48%, and particulate matter by 47% (17). When comparing B20 to diesel fuel, the total unburned hydrocarbons are reduced by 21%, carbon monoxide by 11%, and particulate matter by 10% (17). Finally, only nitrogen oxides (NO_x) are increased with the use of biodiesel. When compared with regular diesel fuel, the use of B100 increases the NO_x emission by 10% and the use of B20 by 2% (17). However, research is being carried out in an attempt to reduce the NO_x emission of biodiesel (18).

Biodiesel Production

Vegetable oils can be used directly in a diesel engine. In such instances, it is said that the engine is running straight vegetable oil (SVO). There are many disadvantages to this practice, including the corrosion caused by fatty acids and the high viscosity of the oil requiring its preheating. Vegetable oils can also be mixed with a solvent such as methanol, ethanol, or butanol to form a microemulsion (19). However, microemulsions are difficult to produce and do not provide consistent results. As a result, these two techniques of using vegetable oils as fuels are not very common.

In order to render vegetable oils much more efficient and useful as fuels, the trigycerides and fatty acids constituting the oils must be chemically altered into more suitable chemicals. This is achieved by thermal cracking (pyrolysis) or transesterification (19). Thermal cracking of vegetable oils produces paraffins and olefins. Not only does this defeat the purpose of burning a cleaner fuel, but also the process is difficult to carry out, quite expensive, and does not lead to a very good throughput; hence it is seldom used (19). Consequently, vegetable oils and animal oils/fats are almost always transformed into biodiesel through transesterification.

Biodiesel is obtained through the bulk transesterification of vegetable oil and animal oil/fat. Any sources of vegetable oil or animal oil/fat can be used, but quite often WFO or WVO are used as raw materials (20). There are two main paths by which it is possible to produce the esters: acid- and base-catalyzed transesterifications (21). On a marginal note, it is also possible to first transform the triglycerides into free fatty acids, which are then esterified or to use enzymes to catalyze the process (21,22).

Both acid- and base-catalyzed processes offer a great yield, usually above 98% (23,24). Nonetheless, in the industrial setting of biodiesel production, base-catalyzed transesterification is preferred to its acid counterpart because it is carried out at low temperature and pressure, is much faster, leads to a minimum number of side reactions, and does not require particularly chemically-resistant materials (23). Figure 1 is an illustration of the conversion process of vegetable oil and animal oil/fat into biodiesel as described by the NBB (21).

Fatty acids are transesterified using a base-catalyzed process. Methanol and a catalyst (typically sodium or potassium hydroxide) are mixed together and placed in contact with the vegetable oil and animal oil/fat (25). Triglycerides are thus transformed into fatty acid methyl esters (FAMEs). After a neutralization step, the glycerin phase (located below the FAME or crude biodiesel phase) is separated from the mixture. Crude biodiesel is purified by washing with warm water. Excess alcohol is then removed from both the crude biodiesel and the glycerin by evaporation or distillation. Methanol is recycled and used again in the process. Glycerin is sometimes purified and sold as pharmaceutical glycerin.

Biodiesel Composition

ASTM International provides a more technical definition of biodiesel than the one presented earlier (11): "a fuel comprised of mono-alkyl esters of long chain fatty acids derived from vegetable
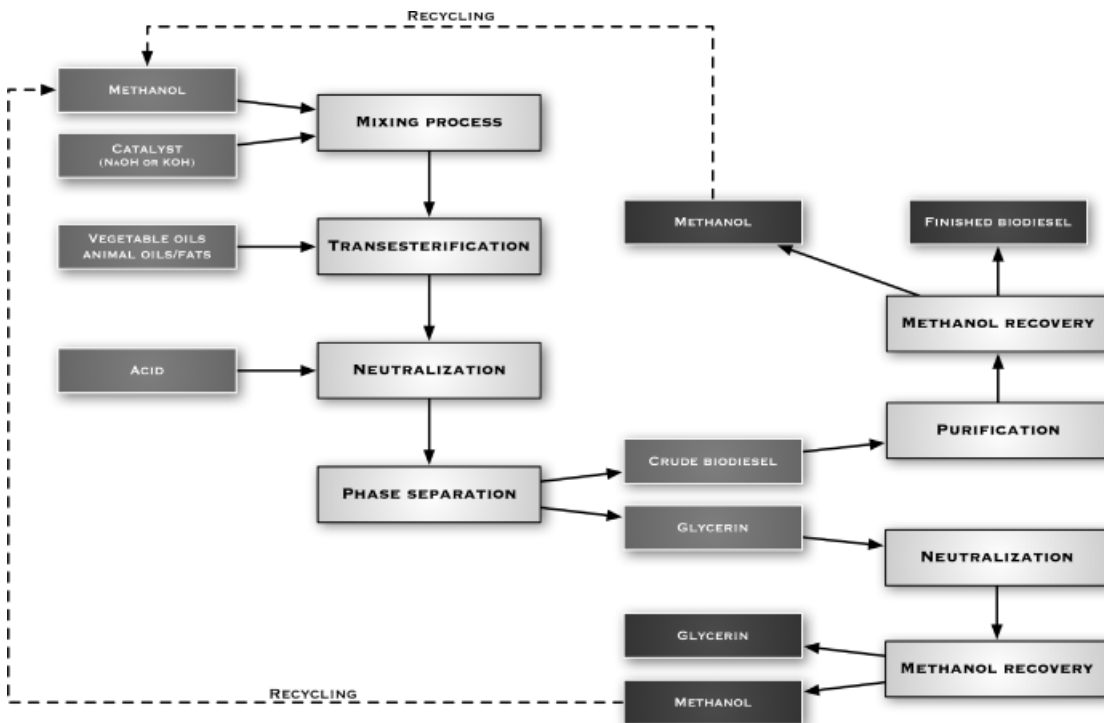

FIG. 1—*Process of conversion of vegetable oil and animal oil/fat into biodiesel as described by the National Biodiesel Board (21).*

TABLE 2—*Typical chemical composition of biodiesels obtained from the transesterification of rapeseed oil and soybean oil into methyl esters (27,28).*

| FAME | Concentration (%) by Mass | |
	Rapeseed Oil-Based Biodiesel	Soybean Oil-Based Biodiesel
C14:0	0.1	N/A
C16:0	4.8	10.8
C16:1	0.2	N/A
C18:0	0.4	4.4
C18:1	61.6	24.1
C18:2	20.6	51.8
C18:3	9.2	6.8
C20:0	0.6	N/A
C20:1	1.4	N/A
C22:0	0.4	N/A
C22:1	0.3	N/A
C24:0	0.1	N/A

Totals do not add up to 100% because compounds in very small proportions are not included. Short names for fatty acids, as shown in the first column, are constituted of a "C" followed by the total number of carbon atoms present in the chain (including the carboxylic group), separated by a colon from the number of carbon-to-carbon double-bonds present in the chain (33).

oils or animal fats, designated B100." This definition summarizes very well the composition of biodiesel: fatty acid esters. Most biodiesel fuels are composed of methyl esters rather than ethyl esters. Testing with ethyl esters has shown that they are perfectly suitable in biodiesel fuel, however the production of methyl esters is less expensive and thus, economically preferred (26).

Hence, biodiesel is typically composed of a series of FAMEs. The exact composition of a given biodiesel (i.e., the nature and proportion of the different FAMEs) is directly dependent on the source oil/fat used to manufacture it. As a result, variation in the ratios and nature of FAMEs do exist. As an illustration, Table 2 shows the typical compositions of two biodiesel samples: one generated from rapeseed oil and one from soybean oil (27,28). One can appreciate the significant difference in the nature and concentration of the different FAMEs. For example, while the most abundant compound in rapeseed biodiesel is methyl octadecenoate or C18:1 ($C_{17}H_{33}COOCH_3$), it is methyl octadecadienaote or C18:2 ($C_{17}H_{31}COOCH_3$) in soybean biodiesel. As a result, the two chromatograms present significantly different patterns. This is contrary to what fire debris analysts are accustomed to see; they expect to see the ratios between the different compounds of a same category (such as the C_3-alkylbenzenes) to be extremely similar among different liquids of the same class.

Biodiesel fuels satisfying European Norm standard 14214 must contain at least 96.5% FAMEs, <0.2% methanol, and <0.05% water (20). The contamination with methanol is an important aspect, particularly from a fire investigation perspective, as the flash point of biodiesel is highly influenced by even a minute amount of residual methanol.

A mixture of FAMEs is quite characteristic of biodiesel, but not exclusive to biodiesel. For example, a product called NoBee-300, manufactured by American Coatings Corporation (Fort-Lauderdale, FL) is composed of more than 90% methyl esters from soybean oil (29,30). This product is sold as a mastic remover and as a general industrial cleaner for greases and oils, adhesives, tars, and asphalts (30). While this product is essentially of the same origin and chemical composition as biodiesel, it is sold under a different commercial application. There may be other "biodiesel" formulations sold under different names and for different applications than as biofuels, although at the time of this article's writing, the

authors are not aware of any. It is also likely that new products of this kind will be developed in the future.

Forensic Approach

When dealing with samples suspected of containing biodiesel or vegetable oil, four basic situations may arise:

- The sample contains regular ignitable liquids or their residues as covered by ASTM standards E1387 and E1618 (31,32).
- The sample contains SVO or its residues.
- The sample contains pure biodiesel or its residues.
- The sample contains a biodiesel blend or its residues.

In the scope of the forensic examination of evidence submitted to the fire debris analyst, there are two possible situations: either the evidence submitted is a neat liquid or it is a (burned or unburned) debris sample.

Neat Liquids

Analyzing neat liquids is always relatively straightforward compared with the analysis of fire debris samples. When the sample contains regular ILR, the fire debris analyst will readily identify the compounds using his or her regular analytical protocol, sometimes based upon ASTM standards E1387 or E1618 (31,32). If the sample contains SVO, triglycerides will likely not be detected by the ILR protocol. Despite that, a few free fatty acids might be present in the chromatogram and may trigger an alarm to the analyst regarding the possible presence of vegetable oil. When analyzing pure vegetable oils, the criminalist must follow procedures developed to analyze vegetable oil residues (VOR) as described in the literature (33,34). When the sample contains pure biodiesel, it is likely that its components will appear in the chromatogram when using the regular analytical protocol for ILR. If a biodiesel blend is present, the chromatogram will exhibit both the diesel fuel and the biodiesel patterns. Figure 2 shows the three chromatograms of diesel fuel, B20, and B100 obtained through a regular ILR protocol with a (5%-phenyl)-methylpolysiloxane phase column. Table 3 shows the oven temperature program used with this particular ILR program.

In the top chromatogram, the pattern of a heavy petroleum distillate (HPD) is readily recognized. The series of *n*-alkanes in a Gaussian shape pattern, along with the presence of pristane and phytane after C_{17} and C_{18}, respectively, and the presence of the cycloalkanes in-between the alkanes are all characteristics of the HPD. The bottom chromatogram, representing B100, introduces new patterns to the fire debris analyst. It contains very few peaks, including an important unresolved cluster between 16 and 17 min. Each significant peak is a FAME as identified in the chromatogram. It is important to keep in mind that each unsaturated FAME presents two or more isomers, depending on the degree of unsaturation (33). The chromatogram in the middle, representing B20, seems to exhibit a low amount of diesel fuel compared with biodiesel, although it contains 80% diesel fuel. This is due to the fact that diesel fuel is composed of several hundred different molecules spread over an important range of boiling points, while biodiesel exhibits only about two dozen peaks.

The chromatographic column used in fire debris analysis, typically equipped with a dimethylpolysiloxane phase or a (5%-phenyl)-methylpolysiloxane phase, is not adapted to perform FAME analysis. This results in a separation of FAMEs that is not optimized as seen in Fig. 2. When analyzing VOR, the analyst must either use a different column, such as a polyalkylene glycol
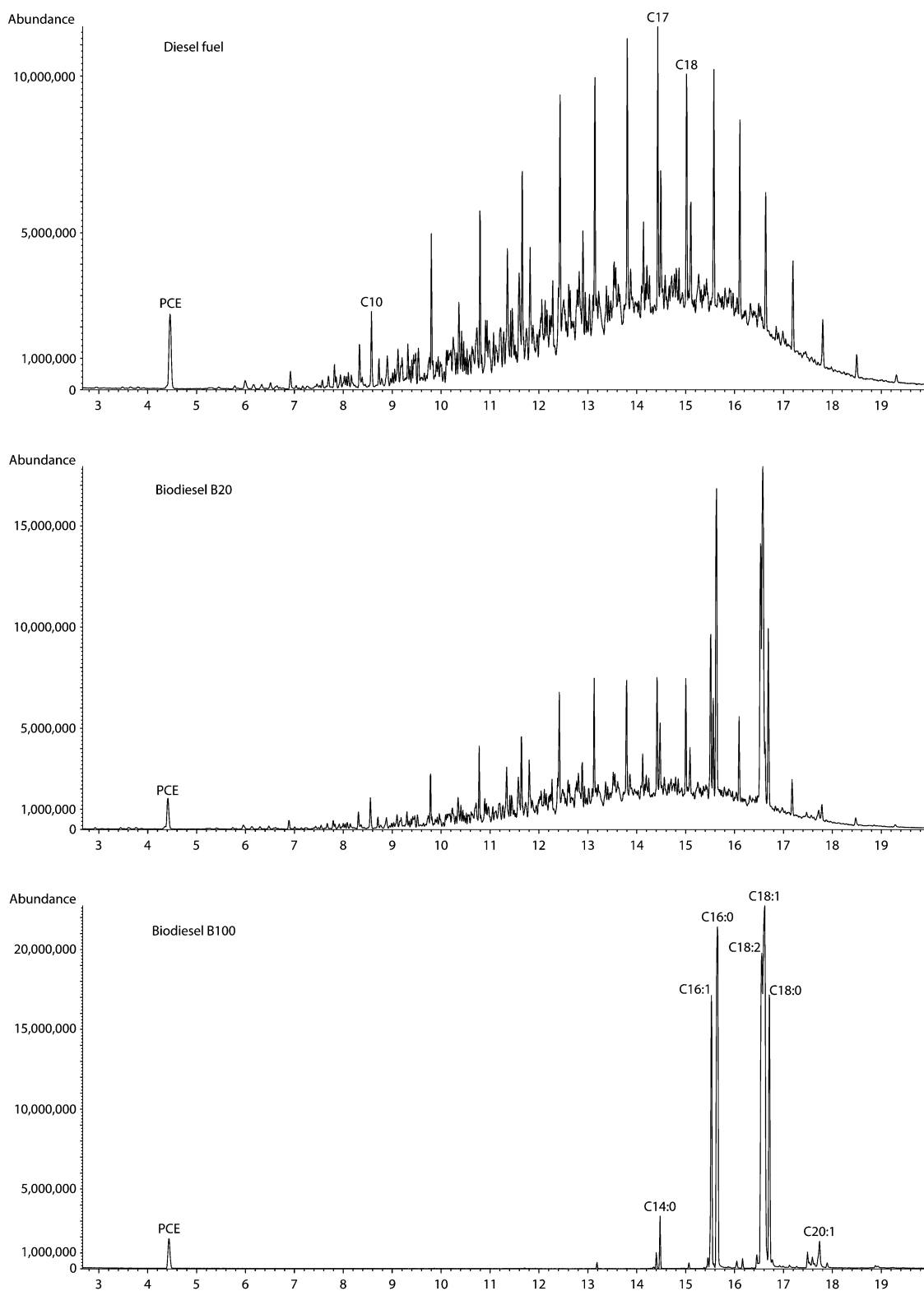
FIG. 2—*Chromatograms obtained using a regular ignitable liquid residues temperature program. Solutions are 2% in dichloromethane. PCE is tetrachloroethylene, the internal standard. Top: diesel fuel. Middle: biodiesel blend (B20). Bottom: biodiesel (B100).*

phase, or develop a specific temperature program (34–37). Unfortunately, the VOR program does not allow for the identification of regular ILR as demonstrated in Fig. 3. Table 3 also presents the temperature parameters used for the VOR protocol.

The top chromatogram shows the end fraction of diesel fuel, ranging from c. C_{15} to C_{21}. Although in this case the presence of

an HPD is suggested, notably through the two pairs of peaks C_{17}/pristane and C_{18}/phytane, it is not possible to identify its presence following the criteria of ASTM standard E1618 (32). Additionally, if ILR lighter than an HPD were present in the sample, it would simply not appear in the chromatogram. The bottom chromatogram, representing B100, shows a slightly better separation of the

TABLE 3—*Oven temperature programs used to analyze ignitable liquid residues (ILR) and fatty acid methyl esters (FAME).*

	ILR	FAME
Initial temperature	60°C for 5 min	220°C for 5 min
Ramp 1	20°C/min to 280°C	0.5°C/min to 225°C
Hold	4 min at 280°C	—
Ramp 2	—	5°C/min to 275°C
Hold	—	5 min at 275°C
Total time (min)	20	30
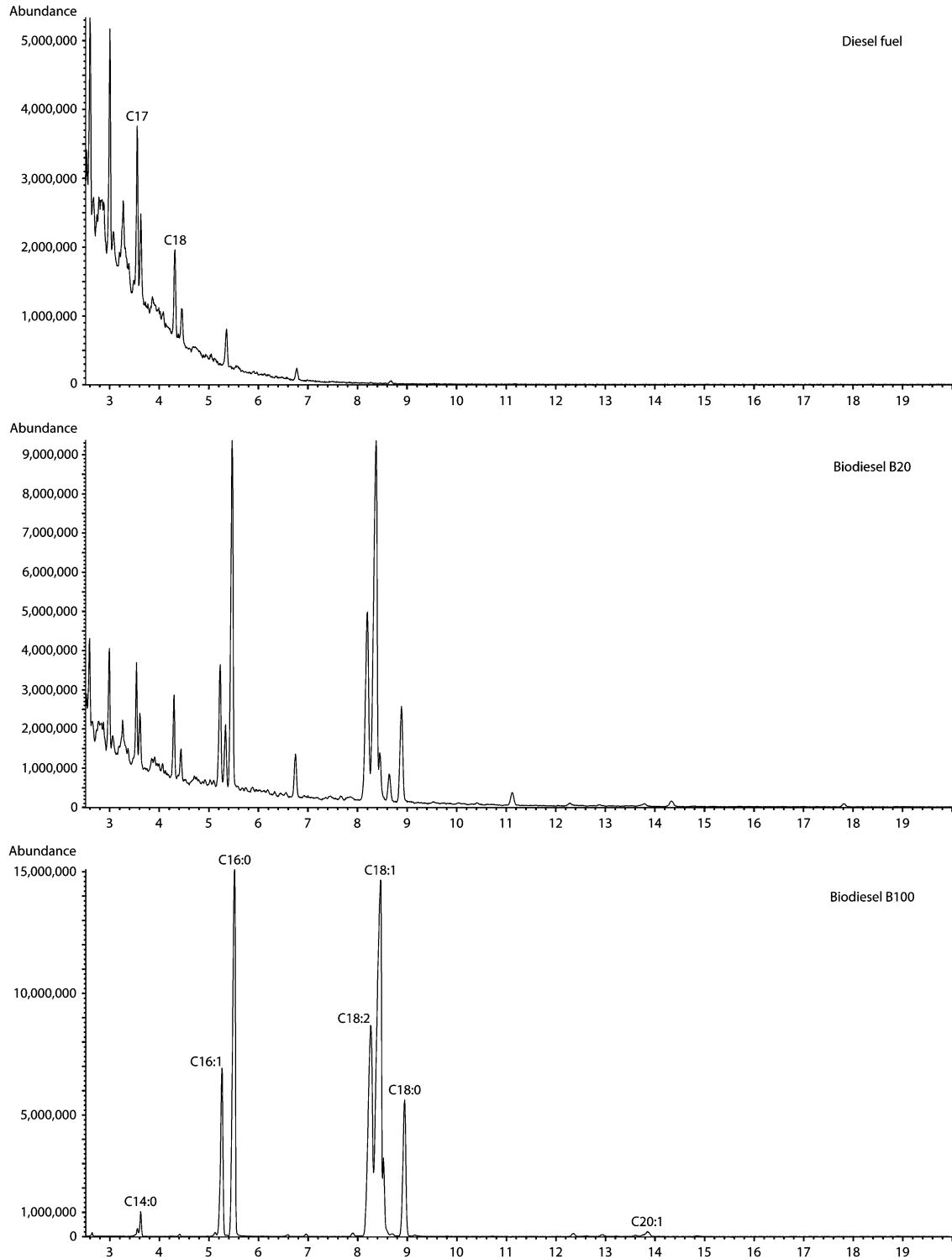
FIG. 3—*Chromatogram obtained using a FAME temperature program designed to analyze vegetable oil residues. Solutions are 2% in dichloromethane. Top: diesel fuel. Note the early eluting peaks, more particularly the two pairs constituted of C_{17}/pristane and C_{18}/phytane. This is the end fraction of diesel fuel. Middle: biodiesel blend (B20). Bottom: biodiesel (B100).*
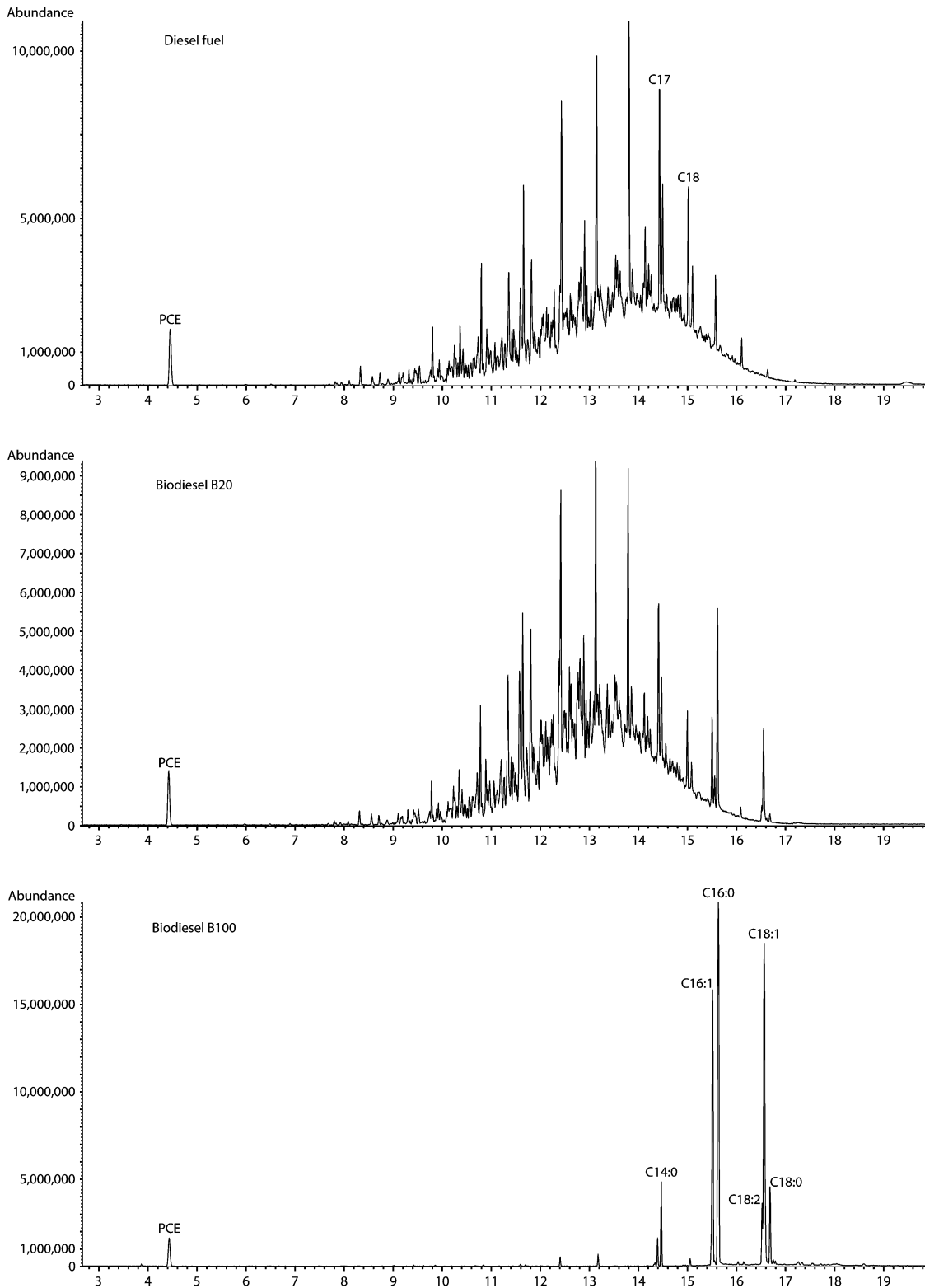
FIG. 4—*Chromatograms of the extracts (passive headspace concentration) obtained using a regular ignitable liquid residues temperature program. PCE is tetrachloroethylene, the internal standard. Top: diesel fuel. Middle: biodiesel blend (B20). Bottom: biodiesel (B100).*

different FAMEs than in Fig. 2. Furthermore, it allows for the separation of heavier FAMEs up to C24:0. In this particular instance, these heavy components are not present in any significant levels. However some VOR or biodiesel may exhibit higher ratios of heavier FAMEs. While the FAME temperature program is more adapted to the analysis of biodiesel, it does not allow for the

proper recognition of ILR. Alternatively, it is also possible to develop a third temperature program that would maximize the separation of both ILR and VOR and permits for their analysis in a single run.

It is important to note that the analysis of biodiesel with a (5%-phenyl)-methylpolysiloxane phase is not the most suitable manner
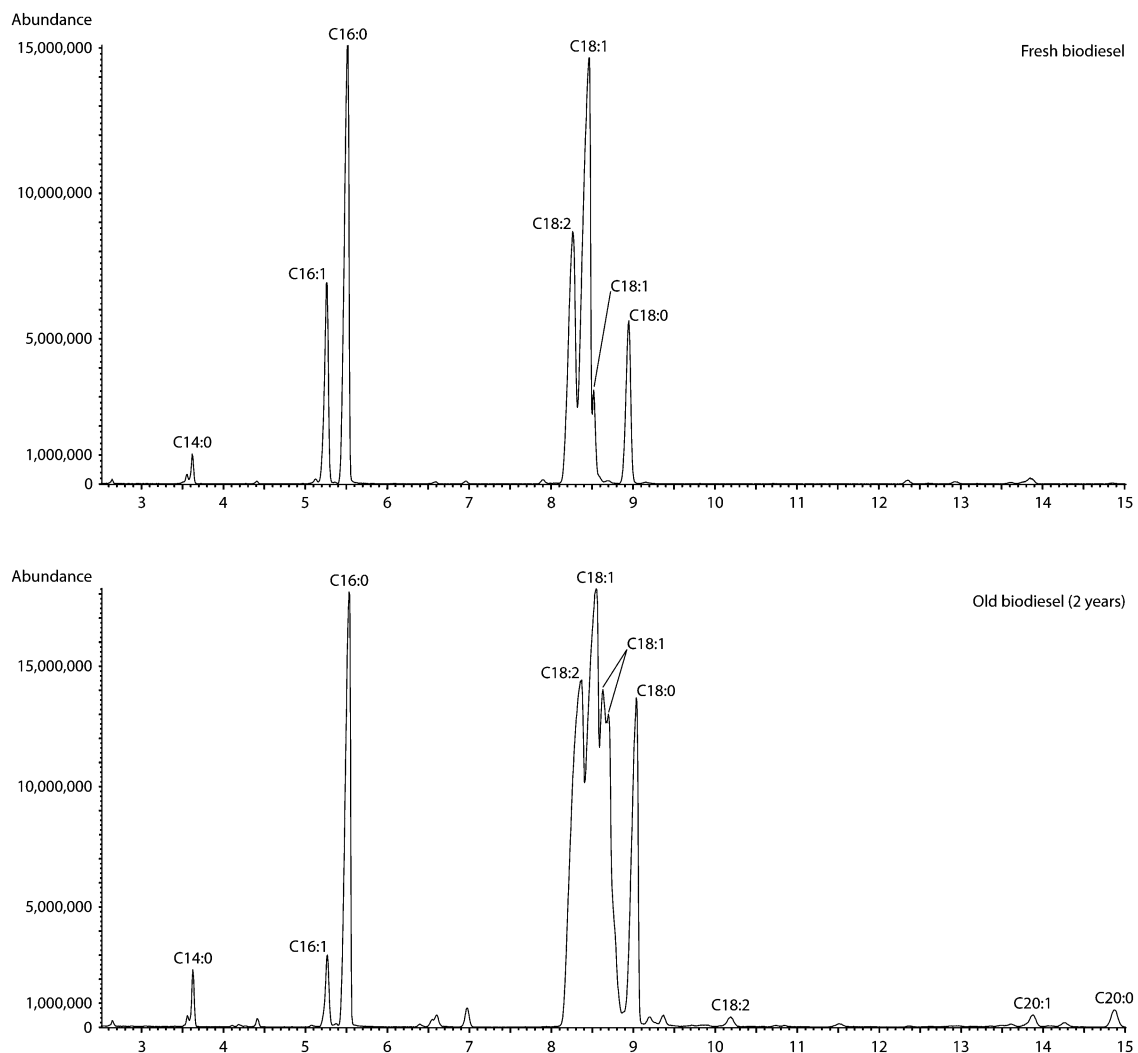
FIG. 5—*Chromatograms of a fresh sample of biodiesel (top) and a sample of biodiesel kept on laboratory shelf in a sealed glass vial for 2 years (bottom). Solutions are 2% in dichloromethane.*

of separating the FAMEs, however it is sufficient from a fire debris analysis perspective when used in conjunction with a mass spectrometer as a detector (34).

Debris Samples

When dealing with debris samples, an extraction of the ILR must be carried out first. VOR are not recovered by passive headspace concentration extraction because they are mostly constituted of triglycerides (34). Fortunately, FAMEs are much more volatile and can be recovered by passive headspace concentration extraction. The resulting analyses of such extracts following a procedure based upon ASTM standard E1412 are shown in Fig. 4 (38). Empty one-quart cans spiked with 10 μL of diesel fuel, B20, and B100, respectively, were extracted for *c.* 16 h at 80°C on a third of an activated charcoal strip (Albrayco Technologies, Cromwell, CT). Each strip was then desorbed with 0.5 mL of dichloromethane.

The usual distortion effect due to the different vapor pressures of the compounds is seen in the top chromatogram with diesel fuel. The heavy compounds, starting around C17 are not as intense as in the neat liquid (compared with Fig. 2). Nevertheless, the early-eluting compounds are also less represented due to a slight displacement effect on the strip. Fortunately, biodiesel is readily recovered as demonstrated in the bottom chromatogram. The difference in ratio due to the different vapor pressures of the compounds is even more pronounced in this instance. Some peaks, such as C18:2 and methyl octadecanoate or C18:0 ($C_{17}H_{35}COOCH_3$), are extremely reduced in the chromatogram and almost everything after C18:0 is missing. When in presence of the mixture B20, the presence of biodiesel may be overlooked as shown in the middle figure. In such instances, it is crucial that the fire debris analyst identifies every peak extraneous to the HPD pattern. The identification of FAMEs should trigger an alarm as to the presence of biodiesel. When dealing with a sample containing biodiesel, while it is normally feasible to identify it by extracting the sample with passive headspace concentration only, it is recommended to also extract the sample using a nonpolar solvent such as pentane or hexane such as described in ASTM standard E1386 (39).

Aging

Biodiesel is not as stable as petroleum products. In practice, it is not recommended that biodiesel be stored for periods exceeding 6 months, unless particular storage measures have been taken and

testing of the fuel's degradation is in place (11). The exact effects of long-term storage of biodiesel are still being evaluated. It appears, however, that degradation products are formed, lowering the quality of the liquid as fuel. As an example, Fig. 5 shows the two chromatograms of the same biodiesel sample at different ages.

The top chromatogram is a fresh sample of biodiesel. The bottom one is a sample *c.* 2 years old that has been stored at room temperature, on a laboratory shelf in a sealed glass vial, away from light. Notice how the clusters of peaks (C18:1 and C18:2) between 8 and 9 min become much less resolved and wider in the bottom chromatogram. It appears that new isomers of C18:1 and C18:2 have appeared in the sample, slightly modifying its pattern.

Conclusion

Biodiesel is an alternative fuel with an increasing presence on the market. It is readily available to the public and eventually to the criminals seeking a liquid accelerant, even though it would not make a good one. It is important for the analyst to have a basic knowledge of its composition and characteristics. Biodiesel is a mixture of FAMEs and can also be blended with diesel fuel. It is readily identified by gas chromatography-mass spectrometry. It can be extracted from fire debris samples by passive headspace concentration on activated charcoal strips, however a solvent extraction using a nonpolar solvent is more suitable for this type of fluid. The criminalist must be careful not to miss a possible mixture with other ILR when interpreting the results of such analyses.

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