REVIEW

Analyzing Biodiesel: Standards and Other Methods

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ABSTRACT: Biodiesel occupies a prominent position among the alternatives to conventional petrodiesel fuel owing to various technical and economic factors. It is obtained by reacting the parent vegetable oil or fat with an alcohol (transesterification) in the presence of a catalyst to give the corresponding monoalkyl esters, which are defined as biodiesel. Because of the nature of the starting material, the production process, and subsequent handling, various factors can influence biodiesel fuel quality. Fuel quality issues are commonly reflected in the contaminants or other minor components of biodiesel. This work categorizes both the restricted species in biodiesel and the physical properties prescribed by the standards, and details the standard reference methods to determine them as well as other procedures. Other aspects of biodiesel analysis, including production monitoring and assessing biodiesel/petrodiesel blends, are also addressed. The types of analyses include chromatographic, spectroscopic, physical properties-based, and wet chemical methods. The justifications for specifications in standards are also addressed.

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Biodiesel (1,2), defined as the monoalkyl esters of vegetable oils or animal fats, is steadily gaining interest and significance in light of recent developments such as the upsurge in petroleum prices and the implementation of financial incentives for its use. With the increasing interest and use, the assurance of fuel properties and quality has become of paramount interest to the successful commercialization and market acceptance of biodiesel. Accordingly, biodiesel standards have been established or are being developed in various countries and regions around the world, including the United States (ASTM D 6751), Europe (EN 14214), Brazil, South Africa, Australia and elsewhere. This article details the specifications in biodiesel standards in ASTM D 6751 and EN 14214, the standards commonly used as reference or base for other standards, and their analysis. Table 1 lists the specifications in the ASTM biodiesel standard, and Table 2 gives corresponding information for the European standards, both for diesel fuel and for heating oil use. For some specifications, alternative methods may be used as discussed in the standards.

Biodiesel can also be used as heating oil. Accordingly, a separate standard (EN 14213) exists in Europe for biodiesel that is to be used as heating oil. The specifications of EN 14213 are also contained in Table 2. A brief discussion of the differences in the specifications of EN 14213 vs. EN 14214 can be found at the end of this article.

Biodiesel is produced by transesterifying the parent oil or fat with an alcohol, usually methanol, in presence of a catalyst, usually a strong base such as sodium or potassium hydroxide, or, preferably and increasingly more commonly, alkoxides. The resulting product therefore can contain not only the desired alkyl ester product but also unreacted starting material (TAG), residual alcohol, and residual catalyst. Glycerol is formed as by-product and separated from biodiesel in the production process, however, traces thereof can be found in the final biodiesel product. Since transesterification is a stepwise process, MAG and DAG formed as intermediates can also be found in biodiesel. Accordingly, these aspects have been addressed in biodiesel standards. The analysis, fuel quality, and production monitoring of biodiesel have been discussed (see articles in Refs. 1–5), but not all aspects of standards were considered, especially in light of their recent adoption, various aspects were categorized differently and new developments have occurred.

Besides these aspects, other issues need to be considered, such as fuel and physical properties as well as storage and handling issues. For example, biodiesel can absorb a certain amount of water during storage. Another example is the susceptibility, of linoleic and linolenic acid esters especially, to oxidation. Other storage parameters also can affect fuel quality. Such issues are also addressed in biodiesel standards.

Some specifications in biodiesel standards are carryovers from petrodiesel standards. However, not all test methods carried over from petrodiesel standards into biodiesel standards are well suited for biodiesel analysis. To account for the nature of biodiesel, many different specifications related to the items just discussed have been introduced into standards. There are often methods that have been developed by oleochemical associations and societies, such as the American Oil Chemists' Society, that may be more suitable.

The present article categorizes biodiesel analysis according to the nature of the materials or properties to be analyzed. Although the standard methods used for analyzing the various specifications will be mentioned, emphasis is placed on methods discussed in the scientific literature. Some specifications in biodiesel standards are straightforward and there is no or only very little discussion in the scientific literature when relating them to biodiesel.

Since potential contaminants of biodiesel can arise during the transesterification reaction, it is important for biodiesel producers to be able to monitor the status of biodiesel production

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TABLE 1
ASTM Biodiesel Standard D 6751[a]

Property	Test method	Limits	Units
Flash point (closed cup)	D 93	130.0 min	°C
Water and sediment	D 2709	0.050 max	% volume
Kinematic viscosity, 40°C	D 445	1.9–6.0	mm^2/s
Sulfated ash	D 874	0.020 max	% mass
Sulfur	D 5453	0.0015 max (S15)	
		0.05 max (S500)	% mass
(ppm)			
Copper strip corrosion	D 130	No. 3 max	
Cetane number	D 613	47 min	
Cloud point	D 2500	Report	°C
Carbon residue	D 4530	0.050 max	% mass
Acid number	D 664	0.50 max	mg KOH/g
Free glycerin	D 6584	0.020	% mass
Total glycerin	D 6584	0.240	% mass
Phosphorus content	D 4951	0.001 max	% mass
Sodium/potassium	UOP 391	5 max. combined	ppm
Distillation temperature, atmospheric equivalent temperature, 90% recovered	D 1160	360 max	°C

[a]The limits are for Grade S15 and Grade S500 biodiesel, with S15 and S500 referring to maximum sulfur specifications (in ppm).

in order to recognize and correct any problems at an early stage. Accordingly, this article also summarizes results on monitoring of the transesterification reaction.

The use of biodiesel/petrodiesel blends also has been increasing significantly. Therefore, the verification of blend levels is another important aspect of biodiesel analysis. Different

TABLE 2
European Biodiesel Standards EN 14214 for Vehicle Use and EN 14213 for Heating Oil Use

Property	Test method	Limits		Unit
		EN 14214	EN 14213	
Ester content	EN 14103	96.5 min	96.5 min	% (mol/mol)
Density; 15°C	EN ISO 3675, EN ISO 12185	860–900	860–900	kg/m^3
Viscosity; 40°C	EN ISO 3104, ISO 3105	3.5–5.0	3.5–5.0	mm^2/s
Flash point	EN ISO 3679	120 min	120 min	°C
Sulfur content	EN ISO 20846; EN ISO 20884	10.0 max	10.0 max	mg/kg
Carbon residue (10% distillation residue)	EN ISO 10370	0.30 max	0.30 max	% (mol/mol)
Cetane number	EN ISO 5165	51 min	—	
Sulfated ash	ISO 3987	0.02 max	0.02 max	% (mol/mol)
Water content	EN ISO 12937	500 max	500 max	mg/kg
Total contamination	EN 12662	24 max	24 max	mg/kg
Copper strip corrosion (3h, 50°C)	EN ISO 2160	1	—	degree of corrosion
Oxidative stability, 110°C	EN 14112	6.0 min	4.0 h	h
Acid value	EN 14104	0.50 max	0.50 max	mg KOH/g
Iodine value	EN 14111	120 max	130 max	g I_2/100 g
Linolenic acid content	EN 14103	12.0 max	—	% (mol/mol)
Content of FAME with ≥4 double bonds		1 max	1 max	% (mol/mol)
Methanol content	EN 14110	0.20 max	—	% (mol/mol)
MAG content	EN 14105	0.80 max	0.80 max	% (mol/mol)
DAG content	EN 14105	0.20 max	0.20 max	% (mol/mol)
TAG content	EN 14105	0.20 max	0.20 max	% (mol/mol)
Free glycerine	EN 14105, EN 14106	0.020 max	0.02 max	% (mol/mol)
Total glycerine	EN 14105	0.25 max	—	% (mol/mol)
Group I metals (Na + K)	EN 14108, EN 14109	5.0 max	—	mg/kg
Group II (Ca + Mg)	prEN 14538	5.0 max	—	mg/kg
Phosphorus content	EN 14107	10.0 max	—	mg/kg
Cold filter plugging point	EN 116	—		°C
Pour point	ISO 3016	—	0 max	°C
Heating value	DIN 51900-1			
	DIN 51900-2			
	DIN 51900-2	—	35 min	MJ/kg

methods for various situations have been developed, including detection of the blend level during use in an engine. Therefore, this article will also deal with blend level detection.

RESULTS AND DISCUSSION

Analysis of production-related and "natural" biodiesel contaminants. During the transesterification process, intermediate MAG and DAG are formed, small amounts of which can remain in the final biodiesel product. Besides these partial glycerols, unreacted TAG as well as unseparated glycerol, FFA, residual alcohol, and catalyst can contaminate the final product. The contaminants can lead to severe operational problems when using biodiesel, such as engine deposits, filter clogging, or fuel deterioration. Therefore, standards such as those in Europe (EN 14214; EN 14213 when using biodiesel for heating oil purposes) and the United States (ASTM D 6751) limit the amount of contaminants in biodiesel fuel. These items and others are discussed in the following text. Each specification in biodiesel standards has been assigned to a specific category, although in some cases other categorizations may also be acceptable.

(i) Glycerol and glycerol esters. Free and total glycerol. Various acylglycerols. Ester content. Both GC and HPLC analyses and combinations thereof have been reported for biodiesel. Generally, GC has been the most widely used method for the analysis of biodiesel owing to its generally higher accuracy in quantifying minor components. However, accuracy of GC analyses can be influenced by factors such as baseline drift, overlapping signals, and aging of standards and samples. Such factors may not always be addressed in standards and reports. Gel permeation chromatography (GPC) as an analytical tool for analysis of transesterification products also has been reported. To date, most chromatographic analyses have been applied to methyl esters and not to higher esters such as ethyl and isopropyl. Most methods would likely have to be modified to analyze the higher esters properly. For example, when using GC, temperature program changes or other alterations may be necessary. The original work (6) on GC analysis reported the investigation of methyl and butyl esters of soybean oil. Not all individual components were separated there in the analysis of butyl soyate, but classes of compounds were analyzed. HPLC analysis was applied to some ethyl, isopropyl, 2-butyl, and isobutyl esters of soybean oil and tallow (7).

To meet the requirements of biodiesel standards, the quantification of individual compounds in biodiesel is not necessary, but the quantification of classes of compounds is. For example, for the determination of MAG, DAG, or TAG (in European standards), it does not matter which FA is (are) attached to the glycerol backbone. For the determination of total glycerol, it does not matter which kind of acylglycerol (MAG, DAG, or TAG) or free glycerol the glycerol stems from as long as the limits of the individual acylglycerol species or free glycerol are observed. That acylglycerols are quantifiable as classes of compounds by GC is a result of the method.

The specifications regarding glycerol esters are analyzed by GC using an FID in both ASTM D 6751 and EN 14214. ASTM D 6751 uses ASTM D 6584, whereas there are several specifications in EN 14214 using GC-based methods. Methyl heptadecanoate is a common standard for FA components, although the stability of standard solutions is an issue (8); freshly prepared solutions should be used, and pyridine may be more suitable as solvent than heptane. These aspects have found the most attention in the scientific literature, and the GC methods in standards are often based on this literature.

The standard reference method EN 14103, for determining ester content in EN 14214, is a GC method utilizing a 30-m CARBOWAX (or comparable) column for determining FA profile. It therefore also serves for the determination of methyl linolenate as discussed below. However, methyl heptadecanoate used as standard presents a problem when using animal fat-based biodiesel because of the latter's natural content thereof (8). Also, the GC temperature program of EN 14103 requires modification for biodiesel containing shorter-chain esters because otherwise erroneous results are obtained for these species (8).

ASTM D 6584 and EN 14105 are based on the same literature results (discussed below). Both use high-temperature (up to 400°C) capillary columns. ASTM D 6584 specifies (5% phenyl)polydimethylsiloxane columns of 10 or 15 m length with 0.32 mm inner diameter and 0.1 mm film thickness. EN 14105 allows for 10-m columns of either 100% dimethylpolysiloxane or 5% diphenylpolysiloxane with the same inner diameter and film thickness. The temperature programs are similar, too, starting out at 50°C and ending at 380 or 370°C. Both methods use a cool on-column injector. Anecdotal evidence suggests that the method used in the standards D 6584 and EN 14105 is suitable only for methyl esters, with quantification problems being encountered with ethyl esters.

However, the first report on chromatographic analysis of transesterification used TLC with FID (TLC/FID; Iatroscan instrument) (9). In another report (10), TLC/FID was used to correlate bound glycerol content with acyl conversion, as determined by GC. It was found in this work that if acyl conversion to methyl esters is >96%, then the amount of bound glycerol is <0.25 wt%; however, it is not clear how the difference in percentage is accounted for. Although the TLC/FID method is easy to learn and use (9), it has been largely abandoned because of lower accuracy, material inconsistencies, sensitivity to humidity (9), and the relatively high cost of the instrument (10).

The first report on the use of capillary GC discussed the quantification of esters as well as MAG, DAG, and TAG (6). The samples were reacted with N,O-bis(trimethylsilyl)trifluoracetamide (BSTFA) to give the corresponding trimethylsilyl (TMS) derivatives of the hydroxy groups. Such derivatizations were carried out in subsequent papers on GC quantification of biodiesel. The TMS derivatives improve the chromatographic properties of the hydroxylated materials and, in case of coupling to a mass spectrometer, facilitate interpretation of their mass spectra. Although originally a short (1.8 m) fused-silica (100% dimethylpolysiloxane) capillary column was used (6), in other work fused-silica capillary columns coated with a 0.1

mm film of (5% phenyl)methylpolysiloxane of 10, 12, or 15 m length typically were used. An analysis of rapeseed ethyl esters was carried out on a GC instrument equipped with an FID and a 1.8 m × 4 mm i.d. packed column (11).

GC analyses usually deal with determination of a specific contaminant or class of contaminants in methyl esters. The original report on biodiesel GC analysis (6) quantified MAG, DAG, and TAG in methyl soyate on a short 100% dimethylpolysiloxane column (1.8 m × 0.32 mm i.d.). Related reports on the quantification of glycerol and acylglycerols exist (12–15). The individual or combined determination of other potential contaminants such as free glycerol or methanol also has been reported.

Most reports on the use of GC for biodiesel analysis use FID. The use of mass spectrometric detectors would eliminate any ambiguities about the nature of the eluting materials since mass spectra unique to individual compounds would be obtained, although quantification may be affected. Two papers exist in the literature in which the use of MS detection is described (12,13). In the determination of free glycerol in biodiesel by GC–MS, selected ion monitoring (SIM) mode was used to track the ions *m/z* 116 and 117 of bis-O-trimethylsilyl-1,4-butanediol (from silylation of the 1,4-butanediol standard) and *m/z* 147 and 205 of tris-O-trimethylsilyl-1,2,3-propanetriol (from silylation of glycerol). The detection limit was also improved for rapeseed methyl ester when using MS in SIM mode (10–5%) compared with the FID detector (10–4%) (12). In extension of this work, the simultaneous detection of methanol and glycerol by MS in SIM mode was reported (13). For detection of (silylated) methanol (trimethylmethoxysilane), peaks at *m/z* 59 and 89 were monitored as were peaks at *m/z* 75 and 103 of the additional (silylated) standard ethanol (trimethylethoxysilane). MS in SIM mode has the additional advantage that interfering signals can be avoided and thus the use of shorter columns is possible (13).

Other authors have also reported the determination of glycerol (16) or methanol (17). Methanol was analyzed using the same GC equipment as in the previous determination of glycerol with only a modification of the oven temperature program. Ethanol was used as a standard for response factor determination. The flash points of biodiesel from palm oil and methanol content were correlated. Underivatized glycerol was detected with 1,4-butanediol as a standard on a short, 2-m glass column (i.d. 4 mm) loaded with Chromosorb 101 (16) whereas the other method used derivatization and a 60 m × 0.25 mm i.d., film 0.25 μm (5% phenyl)methylpolysiloxane column and is reportedly more sensitive (12). The temperature program varied (lower starting temperature when determining methanol) (12,13), but the column was the same.

A further extension of the aforementioned work is the simultaneous determination of glycerol MAG, DAG, and TAG by GC (18). The simultaneous determination of glycerol and acylglycerols in biodiesel has led to the development of corresponding standard reference methods such as ASTM D 6584 and EN 14105, which in turn are included as parameters in full biodiesel standards. Here (18) and in previous work (15), 10 m (5% phenyl)methylpolysiloxane columns with 0.1 mm film (0.25 mm i.d. in Ref. 13; 0.32 mm i.d. in Ref. 18) were used. Major differences were the lower starting temperature of the temperature program (15) and the addition of a standard (1,2,4-butanetriol) for the glycerol analysis. Tricaprin is used as standard for acylglycerol analysis. A cool on-column injector/inlet was used (15,18). The sequence of analyzed compounds eluting from the column in ASTM D 6584 and EN 14195 is glycerol (derivatized), butanetriol standard, methyl esters, MAG (derivatized), tricaprin standard, DAG (derivatized), and, finally, TAG with a total run time of about 31 min. Numerous smaller peaks are visible in the chromatograms, which can likely be traced to minor components such as sterols.

A general advantage of HPLC compared with GC is that usually time- and reagent-consuming derivatizations are not necessary, thereby reducing analysis times. Nevertheless, there are fewer reports of HPLC applied to biodiesel than GC analysis. LC was found to be operationally superior to GC because of the aforementioned reasons, and it was directly applicable to most biodiesel fuels (19). With one exception, ANOVA showed that there was no statistical difference in bound glycerol determination between HPLC and GC. The first report on the use of HPLC (20) described the use of an isocratic solvent system (chloroform with an ethanol content of 0.6%) on a cyano-modified silica column coupled to two GPC columns with density detection. This system allowed for the detection of MAG, DAG, and TAG as well as methyl esters as classes of compounds. The system was useful for quantifying various degrees of conversion of the transesterification reaction.

Other analytical methods for glycerol. An enzymatic method for analyzing glycerol in biodiesel was described to test for completeness of the transesterification reaction (21). Solid-phase extraction of the reaction mixture with subsequent enzymatic analysis was applied. This method was originally intended as a simple method for glycerol determination, but reproducibility and complexity concerns exist (16,22). Recently, an enzymatic method for determining free and total glycerol leading to spectrophotometric detection of a quinonimine dye was commercially available (23), but it is no longer offered by the vendor.

The periodate oxidation of glycerol leads to formaldehyde. Further reaction of formaldehyde with acetylacetone in presence of ammonium acetate (Hantzsch reaction) gives quantifiable 2,6-dimethyl-3,5-diacetyl-1,4-dihydropyridine (3,5-diacetyl-1,4-dihydrolutidine). This derivative has a strong absorption at 410 nm that can then be used for glycerol quantification in biodiesel (24).

Reversed-phase HPLC (25) was used with different detection methods [UV detection at 205 nm, ELSD, and atmospheric pressure chemical ionization-MS (APCI-MS) in positive-ion mode]. Two gradient solvent systems, one consisting of mixing methanol (A) with 5:4 2-propanol/hexane (B) from 100% A to 50:50 A/B (a nonaqueous reversed-phase solvent system), the other of mixing water (A), acetonitrile (B), and 5:4 2-propanol/hexane (C) in two linear gradient steps (30:70 A/B at 0 min, 100% B in 10 min, 50:50 B/C in 20 min, and finally, isocratic 50:50 B/C for 5 min), were applied. The first solvent system was developed for rapid quantification of the transesterifi-

cation of rapeseed oil with methanol by comparing the peak areas of methyl esters and TAG. The contents of individual acids (using normalized peak areas) were subject to error, and the results differed for the various detection methods. The sensitivity and linearity of each detection method varied with the individual TAG. APCI-MS and ELSD had decreased sensitivity with increasing number of double bonds in the FAME, whereas UV will not quantify the saturates. APCI-MS was stated to be the most suitable detection method for the analysis of rapeseed oil and biodiesel. An HPLC-MS-APCI method has been briefly reviewed with respect to its applicability to biodiesel analysis (26).

The use of GPC for the analysis of transesterification products also has been described (27). With a refractive index detector and THF as mobile phase, MAG, DAG, and TAG as well as the methyl esters and glycerol were analyzed. The method was tailored for palm oil, and standards were selected accordingly. Reproducibility was good with the SD at different rates of conversion being 0.27–3.87%.

The combination of LC with GC has also been reported. The purpose of the combination of these two methods is to reduce the complexity of the gas chromatograms and to obtain more reliable peak assignments (28). A fully automated LC-GC instrument was used in the determination of acylglycerols in vegetable oil methyl esters (28). Hydroxy groups were acetylated, and then the methyl esters (sterols and esterified sterols elute with methyl esters) and acylglycerols were pre-separated by LC (variable wavelength detector). The solvent system for LC was hexane/methylene chloride/acetonitrile 79.97:20:0.05. GC (FID) was performed on a 10-m (5% phenyl)methylpolysiloxane column. One LC-GC run required 52 min.

Restrictions on the FA profile. The reason for restrictions on the FA profile, contained mainly in EN 14214, is to exclude components of biodiesel with less desirable properties, for example, with respect to oxidative stability. In practice, this can amount to excluding certain feedstocks for biodiesel production.

(i) Linolenic acid methyl ester content. The content of methyl linolenate is restricted in EN 14214 because of the propensity of methyl linolenate to oxidize. However, the limit (12%) is set so as not to exclude high-oleic rapeseed oil, the major biodiesel source in Europe, as feedstock. The method EN 14103 used for this determination is the same as used for ester content.

(ii) Content of FAME with ≥4 double bonds. This specification serves to eliminate fish oil as biodiesel feedstock. With their even higher content of methylene-interrupted double bonds, fish oil FA are even more prone to oxidation than linolenic acid and its esters.

(iii) Iodine value (IV). IV is a measure of total unsaturation of a lipidic material. The standard method EN 14111 in the European biodiesel standard is based on the classic wet chemical method (Wijs) for determining the IV. It purportedly serves a similar purpose in EN 14214 as do the restrictions on methyl linolenate and fish oil esters. The IV of 120 in EN 14214 can serve to restrict certain vegetable oils as biodiesel feedstock, notably soybean oil or sunflower oil. However, the use of the IV is problematic because of the great number of FA compositions giving the same IV (29). IV restrictions can be overcome by the use of higher esters, such as ethyl or propyl, although the FA profile remains unchanged (29). The use of the IV is also rendered superfluous when an oxidative stability specification, as discussed shortly, is included. Susceptibility to oxidation may be better described by indices termed allylic position equivalents and bis-allylic position equivalents (29).

(iv) Kinematic viscosity. This physical property (discussed shortly) can also be used to restrict the FA profile. For example, shorter-chain FA are excluded by the relatively high minimum value for kinematic viscosity in EN 14214. Although the minimum value for kinematic viscosity prescribed in ASTM D 6751 overlaps most petrodiesel fuels, the high minimum kinematic viscosity value for biodiesel prescribed in EN 14214 is higher than that of many petrodiesel fuels, underscoring the feedstock-restrictive nature of the EN 14214 limit. Also, biodiesel fuels derived from used frying oils tend to possess higher viscosity than those from most vegetable oils, owing to their higher content of *trans* FA and saturated, or, more generally speaking, less unsaturated FA. An upper limit of 5 mm^2/s for kinematic viscosity in biodiesel standards may exclude some frying oils as feedstock.

FFA and acid value. The acid value, like kinematic viscosity, is a facile method for monitoring fuel quality. The acid value is contained in ASTM D 6751 using the method ASTM D 664 and in EN 14214 using the method EN 14104. However, D 664, a potentiometric method, possesses mediocre reproducibility (30), a problem mentioned in the method itself. The problem is likely due to the variability of electrodes. ASTM D 974 is a nonaqueous titration using KOH in isopropanol with *p*-naphtholbenzoin as indicator and is suitable even for colored samples. Analytical results were more consistent using ASTM D 974 than with ASTM D 664. Therefore, ASTM D 974 would be the more appropriate method than ASTM D 664 in the biodiesel standard D 6751 (30). EN 14104 is also a titration; however, it uses a dilute ethanolic KOH solution with phenolphthalein as indicator. Other literature related to the acid value includes titration methods for determining the neutralization number (NN) of biodiesel (31). Two methods for determining strong acids and FFA in one measurement were developed. One method, of particular interest, used potentiometry whereas the other used two acid-base indicators (neutral red, phenolphthalein). The potentiometric method was more reliable, and even with the use of two indicators the NN values derived from the titration method are 10–20 relative percent greater than the real acidity of the sample.

Alcohol. (i) Flash point. The flash point specification serves to restrict the amount of alcohol in the biodiesel fuel. The prescribed methods, both of which use a closed-cup flash point tester, are ASTM D 93 in ASTM D 6751 and ISO 3679 in EN 14214 and restrict methanol to a maximum of about 0.1% in the biodiesel fuel.

(ii) Methanol content. EN 14110, contained in EN 14214, can be applied to mixtures containing 0.01 to 0.5% methanol

and is a GC-based method. The sample is heated in a sealed vial at 80°C and after attaining an equilibrium, a defined amount of the gas phase is injected into the GC. 2-Propanol serves as internal standard.

Catalyst and related matters. (i) Sulfated ash. The sulfated ash test (ASTM D 874 in ASTM D 6751; ISO 3987 in EN 14214) is designed for determining sulfated ash from lubricating oils containing various metal-containing additives. Metals that are covered include Ba, Ca, Mg, Na, K, and Sn, although S, P, and Cl can be present in combined form. To carry out this test, the sample is burned fully with only ash and carbon remaining. This residue is treated with sulfuric acid and heated until oxidation of carbon is complete. The ash is cooled, treated again with sulfuric acid and heated to constant weight. An application to biodiesel is obviously determining residual Na or K from the catalyst.

(ii) Carbon residue. The carbon residue test (ASTM D 4504 in ASTM D 6751; ISO 10370 in EN 14214) is designed to indicate the coking tendency of the sample. The amount of carbon residue formed after evaporation and pyrolysis of the weighed petroleum sample is determined. The results correspond to the Conradson Carbon Residue test (ASTM D 189). For an expected test result of less than 0.10%, the sample can be distilled to give a remaining 10% of the original sample, which is the material then to be tested.

(iii) Sodium and potassium. This specification is contained in EN 14214, which uses the standard reference method EN 14108, and was recently added to ASTM D 6751, which uses UOP 391. There is some coverage of this specification by sulfated ash. The methods use atomic absorption spectroscopy (589 nm for Na in EN 14108; 766.5 nm for K in EN 14109). The determination of Ca, Cl, K, Mg, Na, and P in biodiesel by argon-oxygen mixed-gas inductively coupled plasma optical emission spectroscopy (ICP OES) was described in the literature (32), with the detection limits for Na and K in biodiesel after dilution with kerosene reported at 1.6 (Na 588.995 nm), 1.4 (Na 589.592 nm) and 7.1 (K 766.490 nm) μg kg^{-1}.

(iv) Calcium and magnesium. Calcium and magnesium are of concern for soap formation. They may also be found in animal fats as a result of contact with nonlipidic material. This specification is currently contained in EN 14214, but not (yet) in ASTM D 6751, although there is some coverage by sulfated ash. However, magnesium may not react in the same fashion as other elements in the sulfated ash test, which is why in this case sulfated ash test data need to be treated with caution. EN 14538 calls for analyzing Ca and Mg by ICP OES. Ca is determined at 422.673 nm and Mg at 279.553 nm. Other wavelengths are acceptable if they are free from interferences.

Carryover elements (phosphorus, sulfur) from vegetable oils. These elements can be carried over from vegetable oils, for example, from phospholipids present in all vegetable oils or glucosinolates in rapeseed-based biodiesel. It must be ensured that they are not found in "alternative" biodiesel sources, such as used frying oils or animal fats, that can come in contact with extraneous materials containing these elements.

(i) Sulfur. Sulfur, like phosphorus, is a potential catalyst poison. Most biodiesel fuels inherently contain little or no sulfur, except for the possibility in rapeseed oil just mentioned. The specification is important to show that biodiesel will not negatively affect automotive catalyst systems. Although sulfur is covered in D 4951 (used for the ASTM phosphorus specification), in ASTM D 6751 it is determined by D 5453. D 5453 determines sulfur content by UV fluorescence of the sample during its combustion. SO_2 produced during combustion is converted to excited SO_2*. The fluorescence emitted from the excited SO_2* during its return to the stable SO_2 state is detected, with the signal indicating the amount of sulfur in the sample. The method ISO 20846 in EN 14214 uses the same approach. The alternative method ISO 20884 in EN 14214 uses wavelength-dispersive X-ray fluorescence spectrometry. These methods specifically mention their applicability to biodiesel (FAME) neat (ASTM) or in blends up to 5% (ISO).

(ii) Copper strip corrosion. The copper strip corrosion test (ASTM D 130 in ASTM D 6751; ISO 2160 in EN 14214) consists of dipping a strip of copper into the fuel for a specified time and defined temperature and observing the corrosive action of the fuel. It is a test for corrosive sulfur compounds in the fuel. The corrosive action of these sulfur compounds does not necessarily relate to the total sulfur content as described above. The degree of tarnish on the corroded strip correlates to the overall corrosiveness of the fuel sample.

(iii) Phosphorus. Traces of phosphorus, resulting from phospholipids, can remain in vegetable oils after refining. Phosphorus can poison catalysts used for reduction of exhaust emissions. D 4951 (in D 6751) and EN 14107 (in EN 14214) both use ICP atomic emission spectrometry; EN 14107 specifies 178.3 nm or 213.6 nm. D 4951 suggests these wavelengths, as well as 177.51, 214.91, and 253.40 nm for phosphorus. In the literature, the limit of detection for phosphorus was 32 (177.500 nm) and 67 (178.287 nm) mg kg^{-1} when using argon-oxygen mixed-gas ICP-OES (32).

Fuel and physical properties. (i) Kinematic viscosity. The reduction in viscosity is the major reason why alkyl esters of vegetable oils—biodiesel—are used as fuel and not the neat oil. Thus, the limits on this property are in the range of most, but not all, common vegetable oil (methyl) esters and serve to exclude vegetable oils as fuel. The higher viscosity of the neat oil causes operational problems such as engine deposits. The kinematic viscosity values at 40°C for a variety of neat fatty compounds as well the effects of compound structure on viscosity are discussed in the literature (33). ASTM D 6751 prescribes the use of ASTM D 445, and EN 14214 utilizes ISO 3104/ISO 3105, with ISO 3105 being the specifications and operating instructions for the viscometers used in ISO 3104. Kinematic viscosity, like the acid value, is useful in monitoring the fuel quality of biodiesel during storage since it continuously increases with decreasing fuel quality.

(ii) Cetane number. The cetane number is a dimensionless descriptor of the ignition quality of a diesel fuel. It is related to the ignition delay time a fuel experiences on injection into the combustion chamber. Generally, the higher the cetane number, the shorter the ignition delay time is and the higher the propen-

sity of the fuel to ignite. The minimum cetane numbers prescribed in ASTM D 6751 and EN 14214 exceed those in petrodiesel standards. Both ASTM D 6751 and EN 14214 specify methods using a cetane engine, an engine specifically modified for testing cetane number. Alternatives to the cetane engine such as ASTM D 6890 exist, although not for the same range of cetane numbers. Cetane numbers have been compiled in the literature (1,34).

(iii) Cold flow. Cloud point and cold-filter plugging point (CFPP). ASTM standard D 6751 prescribes the use of the cloud point standard reference method D 2500 for assessing the low-temperature properties of biodiesel. No limit is given; rather a "report" is specified. The reason is that the climate conditions in the United States vary considerably and therefore the needs of biodiesel users vary accordingly. The EN 14214 standard does not mention a low-temperature parameter in its list of specifications; however, it discusses the use of a low-temperature filterability test, the CFPP. Each country using EN 14214 can specify certain temperature limits for different times of year depending on climate conditions.

(iv) Density. A density specification is contained in the European standard EN 14214. The purpose is to exclude extraneous material as biodiesel feedstock.

Other specifications. (i) Oxidative stability. Oxidative stability is one of the major issues affecting the use of biodiesel because of its content of esters of linoleic and linolenic acids, whose bis-allylic methylene positions are especially susceptible to oxidation. The Rancimat method, described in EN 14112, has been included in the European biodiesel standards. It is nearly identical to the AOCS Oil Stability Index (OSI) method Cd12b-92 (35). Both methods are automated and involve heating the sample to a specified temperature (usually 110°C) and bubbling air through the sample, which in turn sweeps volatiles from the sample into water. The conductivity of the water is measured as it changes when volatile acids are contained in it. Generally, oxidation is slow initially. The point at which the rate of oxidation increases is the induction period (maximum change of rate of oxidation; second derivative of the conductivity with respect to time) and is the time measured by Rancimat or OSI. These methods are also suitable for investigating the effectiveness of antioxidants. The temperature generally used for the Rancimat or OSI method (110°C) may be considered rather high and not necessarily reflecting real-world conditions. It may also be argued that, with the inclusion of the oxidative stability specification in EN 14214, the iodine value is not necessary in EN 14214.

(ii) Water or water and sediment. Water in the sample can promote microbial growth, lead to tank corrosion, participate in the formation of emulsions, as well as cause hydrolysis or hydrolytic oxidation. Sediment can reduce the flow of oil from the tank to the combustion chamber. D 2709 (water and sediment) in D 6751 prescribes the use of a centrifuge whereas ISO 12937 in EN 14214 represents a coulometric Karl-Fischer titration.

(iii) Total contamination. EN 12662, contained in EN 14214, is a method for determining contamination as the content of undissolved substances in middle distillates (in mg/kg). It applies to liquid petroleum products with a kinematic viscosity <8 mm^2/s at 20°C or <5 mm^2/s at 40°C.

(iv) Distillation temperature. ASTM D 6751 prescribes the use of ASTM D 1160 for determining the distillation curve of the fuel. This is an example of a specification carried over from petrodiesel standards and actually has no application to biodiesel. Biodiesel does not exhibit a distillation curve since the fatty esters comprising it have very similar b.p. under the reduced pressure conditions of this method. For petrodiesel, the distillation curve is associated with properties such as viscosity, vapor pressure, heating value, and average M.W.

Issues not directly addressed in standards. Analysis of other minor components (sterols) in biodiesel. Sterols are nonglyceridic materials in vegetable oils, and traces thereof can therefore be present in biodiesel, in which they are soluble. Thus, they may also influence fuel quality. Accordingly, the GC determination of sterols and sterol esters in biodiesel (36) was conducted, with the method being nearly identical to the other method by the authors (15), the differences being in the use of sterol standards and a slight modification of the GC temperature program to spread the sterol peaks, leading to condensation or overlapping of the peaks of the other classes of compounds. Detection was carried out with an FID and derivatization with N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA, with 1% trimethylchlorosilane) and the column again was a (5% phenyl)methylpolysiloxane capillary column. Sterols identified in the rapeseed-based biodiesel included β-sitosterol, brassicasterol, campesterol, cholesterol, stigmasterol, and 5-avenasterol as well as sterol esters. The total concentration of sterols in rapeseed methyl ester was 0.339–0.500%, and sterol esters was 0.588–0.722%. In another analysis of sterol content in rapeseed methyl ester, the same authors found a sterol content of 0.70–0.81% (36). Other authors (14) also pointed out the presence of sterols and sterol esters in biodiesel.

LC-GC was also applied to the analysis of sterols in biodiesel derived from rapeseed oil (37,38). Five different types of methyl esters were analyzed for sterols by on-line LC-GC (38). The methyl esters were those of rapeseed, soybean, sunflower, high-oleic sunflower, and used frying oil. The sterols were silylated with N-methyl-N-trimethylsilyltrifluoracetamide (MSTFA). No saponification and off-line pre-separation were required. The methyl esters were separated from the sterols by LC with a hexane/methylene chloride/acetonitrile 79.9:20:0.1 solvent system. GC analysis was carried out with a 12 m (5% phenyl)methylpolysiloxane column and FID detection. Total concentrations of free sterols were 0.20–0.35 wt% for the five samples, whereas sterol esters displayed a range of 0.15–0.73 wt%. Soybean oil methyl ester was at the lower end (0.20 and 0.15%, respectively), while rapeseed oil methyl ester was the higher end (0.33 and 0.73%, respectively). In a comparison of two methods, saponification and isolation of the sterol fraction with subsequent GC analysis and LC-GC analysis of sterols in rapeseed oil methyl ester (38), LC-GC was recommended, despite the sophisticated instrumentation required, because of additional information, short analysis

time, and reproducibility. The total sterol content in rapeseed methyl ester was 0.70–0.81 wt%.

Reaction monitoring. (i) Chromatographic and spectroscopic monitoring. Chromatographic analysis was originally applied to analyzing the transesterification reaction (9,10), as already discussed.

Both ^{1}H and ^{13}C NMR have been used for monitoring the transesterification reaction. When using ^{1}H NMR, the protons of the methylene group adjacent to the ester moiety in TAG and the protons in the alcohol moiety of the product methyl esters were used to monitor the yield (39). A straightforward equation,

$$C = 100 \times (2A_{ME}/3A_{\alpha\text{-}CH_2}) \quad [1]$$

in which C is the conversion of TAG feedstock (vegetable oil) to the corresponding methyl ester, A_{ME} is the integration value of the protons of the methyl esters (the strong singlet peak), and $A_{\alpha\text{-}CH_2}$ is the integration value of the methylene protons, gives the conversion of the reaction. The factors 2 and 3 derive from the fact that the methylene carbon possesses two protons and the alcohol (methanol-derived) carbon has three attached protons. Other authors studied the ethanolysis of soybean oil by ^{1}H NMR using the ester ethoxy and glyceridic signals in the range of 4.04–4.40 ppm (40) and compared their results with viscosity and total glycerol determinations. These authors found NMR faster and simpler to use than GC and/or HPLC. However, instrumentation and maintenance costs must also be considered.

Turnover and reaction kinetics of the transesterification of rapeseed oil with methanol were studied by ^{13}C NMR (41) with benzene-d_6 as solvent. The signals at approximately 14.5 ppm of the terminal methyl groups unaffected by the transesterification were used as internal quantification standard. The methyl signal of the produced methyl esters registered at around 51 ppm and the glyceridic carbons of the MAG, DAG, and TAG at 62–71 ppm. Analysis of the latter peak range allowed the determination of transesterification kinetics.

NIR spectroscopy was used to monitor the transesterification reaction (42). The basis for quantification is differences in the NIR spectra at 6005 and at 4425–4430 cm^{-1}, where methyl esters display peaks whereas TAG exhibit shoulders. Ethyl esters could be distinguished in a similar fashion (42). Using the absorption at 6005 cm^{-1} rather than the one at 4425 cm^{-1} gave better quantification results. It appears that ethyl esters, and perhaps even higher esters, may be distinguished similarly by NIR from TAG, but no results have been reported yet. NIR spectra were obtained with the aid of a fiber-optic probe coupled to the spectrometer, which renders their acquisition particularly easy and time-efficient.

While the NIR work just mentioned used a model system to describe monitoring of transesterification and to develop quantification methods, other work applied the method to a transesterification reaction in progress on a 6-L scale. Spectroscopic results were obtained not only by NIR but also by ^{1}H NMR (43). The results of both spectroscopic methods, which can be correlated by simple equations, were in good agreement. Two NMR approaches were used, one being the use of the methyl ester protons (peak at 3.6 ppm) and the protons on the carbons next to the glyceryl moiety (α-CH_2; peaks at 2.3 ppm) (43). The second approach was the use of the methyl ester protons and the protons of the glyceryl moiety (peaks at 4.1–4.3 ppm) in the TAG (43).

Contaminants of biodiesel cannot be fully quantified by NIR at the low levels called for in biodiesel standards. The accuracy of the NIR method in distinguishing TAG and methyl esters is in the range of 1–1.5%, although in most cases better results are achieved. To circumvent this difficulty, an inductive method can be applied. The inductive method consists of verifying by, say, GC, that a biodiesel sample meets standards. The NIR spectrum of this sample would be recorded. The NIR spectrum of the feedstock would also be recorded as well as the spectra of intermediate samples at conversions of, for example, 25, 50, and 75%. A quantitative NIR evaluation method could then be established. If, when conducting another transesterification reaction, the NIR spectrum indicates that the reaction with the same parameters has attained conversion to a product that (within experimental error of NIR) conforms to standards, it can be safely assumed that this result is correct, even if not all potential contaminants have been fully analyzed. Only if a significant deviation is indicated by NIR would a detailed investigation by a more complex method such as GC be necessary. The NIR procedure is faster, easier, and considerably less labor intensive to perform than GC.

The change in absorbance at 1378 cm^{-1} (terminal CH_3 and OCH_2 in acylglycerols) was monitored during biodiesel production by ATR-FTIR (ATR: attenuated total reflectance) (44). Conversion of TAG to FAME involves loss of glycerol, therefore the peak at 1378 cm^{-1} decreases. The results of ATR-FTIR were correlated with GPC. The agreement between both methods was excellent, and they could be considered equivalent. Other authors (45) used the 1300–1060 cm^{-1} spectral region to distinguish methyl esters and TAG. The O–CH_3 peak at 1200 cm^{-1} increases when the percentage of methyl ester increases. The C–CH_2–O vibration at 1100 cm^{-1} is reduced in the methyl esters but is present in TAG. For monitoring the ethanolysis of degummed soybean oil by FTIR, the C=O peak of the ester groups in the range of 1700–1800 cm^{-1} is used (46). When applying principal component analysis to standard mixtures of triolein and ethyl oleate, only two principal components captured 99.95% of the total spectral variance, with a multivariate calibration model subsequently being developed. The results agreed with size exclusion chromatography analysis.

Viscometry. The viscosity difference between component TAG of vegetable oils and their corresponding methyl esters resulting from transesterification is approximately one order of magnitude. The viscosity difference forms the basis of an analytical method, viscometry, applied to determining the conversion of vegetable oil to methyl ester (47). Viscosities determined at 20 and 37.8°C were in good agreement with GC analyses conducted for verification purposes. The viscometric method, especially results obtained at 20°C, is reported to be suitable for process control purposes due to its rapidity (47).

Similar results were obtained from density measurements (47). However, it appears that the viscosity of the final product, which depends on the FA composition, needs to be predicted from the viscosity values of the individual components.

Biodiesel in lubricating oil. Determination of the amount of biodiesel in lubricating oil (48) by mid-IR spectroscopy with a fiber-optic probe has been reported. The problem is significant because biodiesel can cause dilution of the lubricant, which may ultimately result in engine failure. The dilution is attributed to the higher boiling range of biodiesel (48,49) compared with conventional diesel fuel, whose more volatile components have less chance to dilute the lubricant. The mid-IR range used was 1820–1680 cm^{-1}, which is typical for carbonyl absorption and is observed in neither conventional diesel fuel nor the lubricating oil. Previous to this work, other authors had used IR spectroscopy (without the aid of a fiber-optic probe) in the range 1850–1700 cm^{-1} to analyze biodiesel in lubricating oil (49). The carbonyl absorption at 1750 cm^{-1} was not disturbed by the absorption of oxidation products at 1710 cm^{-1}. However, the carbonyl absorptions in the mid-range IR spectra of TAG and FAME are almost identical, and care must be taken that it be known whether TAG or methyl esters are being analyzed. IR spectroscopy can also be used to identify FAME in machinery oils (50).

Biodiesel blends. For direct determination of blend levels of biodiesel with petroleum-based diesel fuel by IR spectroscopy, the peak of the carbonyl moiety at approximately 1740 cm^{-1} was used (51). Partial least squares (PLS) models based on IR or NIR spectra were suitable for identifying blends of 0–5% biodiesel with petrodiesel (52). Principal component analysis of the region 1700–1800 cm^{-1} could distinguish blends of petrodiesel with biodiesel or untransesterified vegetable oil. Blend detection by IR using this peak is the basis of the European standard reference method EN 14078 [Determination of fatty acid methyl esters (FAME) in middle distillates—Infrared spectroscopy method]. Chemometric techniques using PLS and principal component analysis form the basis of the methods using IR or NIR.

In NIR spectroscopy, the peaks used for blend level determination are those also used for monitoring transesterification and fuel quality (53). The use of the NIR range may permit using a spectrometer without any changes in instrument settings for monitoring reaction and fuel quality as well as for determining blend levels. Also, some characteristic peaks of TAG in vegetable oils or animal fats and methyl esters occur at nearly the same wavenumber (1740 cm^{-1}) in the mid-IR while NIR uses differences in the spectra of methyl esters and TAG. Thus, NIR may be able simultaneously to detect whether the petroleum diesel fuel was blended with biodiesel or TAG-containing oil or fat, the latter not being acceptable.

GC is likely less suitable for blend analysis owing to the very complex chromatograms caused by the numerous components of conventional diesel fuel. However, LC using an isocratic 90:10 hexane/methyl *tert*-butylether (MTBE) solvent system has been used for determining blend levels (54). ELSD or UV detectors are suitable; however, the ELSD offers the advantage that it is a mass detector and does not rely on the presence of double bonds as the UV detector does. The method was developed for B1–B30, with precision established by use of standards. The method also can be used for quantifying TAG in petrodiesel. Another paper reports on the simultaneous determination of aromatics and FAME in blends of biodiesel with petrodiesel by HPLC using a refractive index and a UV detector (55).

Silica cartridge chromatography with hexane/diethyl ether as solvents was used to separate biodiesel from conventional diesel fuel, which was then analyzed by GC (56). In a related work, acetylation of the contaminants in a blend was carried out, the blend separated by means of a silica cartridge with hexane as solvent, and then the biodiesel fraction analyzed by GC (57).

Another method for blend level detection of biodiesel uses the saponification value (56). The ester number, which is defined as the difference between the saponification value and the acid value, of blended fuels was determined and the methyl ester fraction determined using an average M.W. of methyl esters (51) If the average M.W. of the biodiesel is unknown, methyl oleate can be used as a reference (51). The ester number method yielded results comparable with those using IR spectroscopy (51).

The on-vehicle analysis of biodiesel blends is necessary to adjust engine settings such as fuel injection timing for improving performance and emissions (58,59) in response to fueling with different blend levels of biodiesel or when refueling with neat biodiesel or petroleum-based diesel fuel in alternating fashions. For the purpose of on-vehicle analysis, a commercial dielectric fuel sensor originally developed for detecting the level of alcohol (methanol or ethanol) in gasoline/alcohol blends (58) was used. The average frequency difference of approximately 7 Hz suffices for use in blend level detection (58). Another suitable sensor, also based on dielectric properties and originally designed for measuring soil humidity and salinity, was developed (59,60). The frequency output of the sensors is linearly proportional to the blend level of biodiesel.

Biodiesel for heating oil use. Biodiesel can also be used as heating oil. A comparison of the two biodiesel standards (Table 2), one for vehicle use (EN 14214) and one for heating oil use (EN 14213) in Europe, reveals that most specifications are the same or very similar. The cetane number, a diesel engine-specific parameter, is, for obvious reasons, not included in the heating oil standard. Since heating oil burners and/or the conditions under which biodiesel is used as heating fuel are less sensitive to some components or contaminants, the heating oil standard does not include the specifications of Group I and Group II metals as well as methanol and phosphorus. Also, the requirement on oxidative stability is more lenient in the heating oil standard. In addition, there is no restriction on the amount of linolenate in the biodiesel fuel, although the iodine value is set at 130, slightly higher than for vehicle use. The heating oil standard contains a pour point specification not found in the standard for vehicle use. The CFPP is listed in EN 14213 but without limits, but it is contained in EN 14214 with geographical and seasonal limits. The heating oil standard also

contains, for obvious reasons, a heating value specification. The heating value specification would let virtually all biodiesel fuels be suitable for heating oil purposes.

REFERENCES

1. Knothe, G., J. Van Gerpen, and J. Krahl (eds.), *The Biodiesel Handbook*, AOCS Press, Champaign, Illinois, 2005, 302 pp.
2. Mittelbach, M., and C. Remschmidt, *Biodiesel—The Comprehensive Handbook*, published by M. Mittelbach, Karl-Franzens-Universität Graz, Graz, Austria, 2004.
3. Knothe, G., Analytical Methods Used in the Production and Fuel Quality Assessment of Biodiesel, *Trans. ASAE 44*:193–200 (2001).
4. Mittelbach, M., Diesel Fuel Derived from Vegetable Oils, VI: Specifications and Quality Control of Biodiesel, *Bioresour. Technol. 56*:7–11 (1996).
5. Komers, K., R. Stloukal, J. Machek, F. Skopal, and A. Komersová, Biodiesel Fuel from Rapeseed Oil, Methanol, and KOH. Analytical Methods in Research and Production, *Fett/Lipid 100*:507–512 (1998).
6. Freedman, B., W.F. Kwolek, and E.H. Pryde, Quantitation in the Analysis of Transesterified Soybean Oil by Capillary Gas Chromatography, *J. Am. Oil Chem. Soc. 63*:1370–1375 (1986).
7. Foglia, T.A., and K.C. Jones, Quantitation of Neutral Lipid Mixtures Using High Performance Liquid Chromatography with Light Scattering Detection, *J. Liq. Chromatogr. Relat. Technol. 20*:1829–1838 (1997).
8. Schober, S., I. Seidl, and M. Mittelbach, Ester Content Evaluation in Biodiesel from Animal Fats and Lauric Oils, *Eur. J. Lipid Sci. Technol. 108*:309–314 (2006).
9. Freedman, B., E.H. Pryde, and W.F. Kwolek, Thin Layer Chromatography/Flame Ionization Analysis of Transesterified Vegetable Oils, *J. Am. Oil Chem. Soc. 61*:1215–1220 (1984).
10. Cvengroš, J., and Z. Cvengrošová, Quality Control of Rapeseed Oil Methyl Esters by Determination of Acyl Conversion, *Ibid. 71*:1349–1352 (1994).
11. Cvengrošová, Z., J. Cvengroš, and M. Hronec, Rapeseed Oil Ethyl Esters as Alternative Fuels and Their Quality Control, *Petrol. Coal 39*:36–40 (1997).
12. Mariani, C., P. Bondioli, S. Venturini, and E. Fedeli, Vegetable Oil Derivatives as Diesel Fuel. Analytical Aspects. Note 1: Determination of Methyl Esters, Mono-, Di-, and Triglycerides, *Riv. Ital. Sostanze Grasse 69*:549–551 (1991).
13. Plank, C., and E. Lorbeer, Quality Control of Vegetable Oil Methyl Esters Used as Diesel Fuel Substitutes: Quantitative Determination of Mono-, Di-, and Triglycerides by Capillary GC, *J. High Resolut. Chromatogr. 16*:609–612 (1992).
14. Mittelbach, M., Diesel Fuel Derived from Vegetable Oils, V [1]: Gas Chromatographic Determination of Free Glycerol in Transesterified Vegetable Oils, *Chromatographia 37*:623–626 (1993).
15. Mittelbach, M., G. Roth, and A. Bergmann, Simultaneous Gas Chromatographic Determination of Methanol and Free Glycerol in Biodiesel, *Ibid. 42*:431–434 (1996).
16. Bondioli, P., C. Mariani, A. Lanzani, and E. Fedeli, Vegetable Oil Derivatives as Diesel Fuel Substitutes. Analytical Aspects. Note 2: Determination of Free Glycerol, *Riv. Ital. Sostanze Grasse 69*:7–9 (1992).
17. Bondioli, P., C. Mariani, E. Fedeli, A.M. Gomez, and S. Veronese, Vegetable Oil Derivatives as Diesel Fuel Substitutes. Analytical Aspects. Note 3: Determination of Methanol, *Ibid. 69*:467–469 (1992).
18. Plank, C., and E. Lorbeer, Simultaneous Determination of Glycerol, and Mono-, Di-, and Triglycerides in Vegetable Oil Methyl Esters by Capillary Gas Chromatography, *J. Chromatogr. A 697*:461–468 (1995).
19. Foglia, T.A., K.C. Jones, A. Nuñez, J.G. Phillips, and M. Mittelbach, Comparison of Chromatographic Methods for the Determination of Bound Glycerol in Biodiesel, *Chromatographia 60*:305–311 (2004).
20. Trathnigg, B., and M. Mittelbach, Analysis of Triglyceride Methanolysis Mixtures Using Isocratic HPLC with Density Detection, *J. Liq. Chromatogr. 13*:95–105 (1990).
21. Bailer, J., and K. de Hueber, Determination of Saponifiable Glycerol in "Bio-Diesel," *Fresenius' J. Anal. Chem. 340*:186 (1991).
22. Lozano, P., N. Chirat, J. Graille and D. Pioch, Measurement of Free Glycerol in Biofuels, *Ibid. 354*:319–322 (1996).
23. Anon., Glycerine, *Chem. Mark. Rep. 263(21)*:12 (May 26, 2003).
24. Bondioli, P., and L. Della Bella, An Alternative Spectrophotometric Method for the Determination of Free Glycerol in Biodiesel, *Eur. J. Lipid Sci. Technol. 107*:153–157 (2005).
25. Holãapek, M., P. Jandera, J. Fischer, and B. Proke‰, Analytical Monitoring of the Production of Biodiesel by High-Performance Liquid Chromatography with Various Detection Methods, *J. Chromatogr. A 858*:13–31 (1999).
26. Holãapek, M., P. Jandera, and J. Fischer, Analysis of Acylglycerols and Methyl Esters of Fatty Acids in Vegetable Oils and Biodiesel, *Crit. Rev. Anal. Chem. 31*:53–56 (2001).
27. Darnoko, D., M. Cheryan, and E.G. Perkins, Analysis of Vegetable Oil Transesterification Products by Gel Permeation Chromatography, *J. Liq. Chromatogr. Relat. Technol. 23*:2327–2335 (2000).
28. Lechner, M., C. Bauer-Plank, and E. Lorbeer, Determination of Acylglycerols in Vegetable Oil Methyl Esters by On-line Normal Phase LC-GC, *J. High Resolut. Chromatogr. 20*:581–585 (1997).
29. Knothe, G., Structure Indices in FA Chemistry. How Relevant Is the Iodine Value? *J. Am. Oil Chem. Soc. 79*:847–854 (2002).
30. Mahajan, S., S.K. Konar, and D.G.B. Boocock, Determining the Acid Number of Biodiesel, *Ibid. 83*:567–570 (2006).
31. Komers, K., F. Skopal, and R. Stloukal, Determination of the Neutralization Number for Biodiesel Fuel Production, *Fett/Lipid 99*:52–54 (1997).
32. Edlund, M., H. Visser, and P. Heitland, Analysis of Biodiesel by Argon-Oxygen Mixed-Gas Inductively Coupled Plasma Optical Emission Spectrometry, *J. Anal. At. Spectrom. 17*:232–235 (2002).
33. Knothe, G., and K.R. Steidley, Kinematic Viscosity of Biodiesel Fuel Components and Related Compounds. Influence of Compound Structure and Comparison to Petrodiesel Fuel Components, *Fuel 84*:1059–1065 (2005).
34. Knothe, G., A. Matheaus, and T.W. Ryan III, Cetane Numbers of Branched and Straight-chain Fatty Esters Determined in an Ignition Quality Tester, *Fuel 82*:971–975 (2003).
35. AOCS, *Official Methods and Recommended Practices of the AOCS,* 5th edn., AOCS Press, Champaign, Illinois, 1997
36. Plank, C., and E. Lorbeer, Analysis of Free and Esterified Sterols in Vegetable Oil Methyl Esters by Capillary GC, *J. High Resolut. Chromatogr. 16*:483–487 (1993).
37. Plank, C., and E. Lorbeer, Minor Components in Vegetable Oil Methyl Esters I: Sterols in Rapeseed Oil Methyl Ester, *Fett Wiss. Technol. 96*:379–386 (1994).
38. Plank, C., and E. Lorbeer, On-line Liquid Chromatography–Gas Chromatography for the Analysis of Free and Esterified Sterols in Vegetable Oil Methyl Esters Used as Diesel Fuel Substitutes, *J. Chromatogr. A 683*:95–104 (1994).
39. Gelbard, G., O. Brès, R.M. Vargas, F. Vielfaure, and U.F. Schuchardt, ^{1}H Nuclear Magnetic Resonance Determination of the Yield of the Transesesterification of Rapeseed Oil with Methanol, *J. Am. Oil Chem. Soc. 72*:1239–1241 (1995).
40. Neto, P.R.C., M.S.B. Caro, L.M. Mazzuco, and M. da Graça Nascimento, Quantification of Soybean Oil Ethanolysis with ^{1}H NMR, *Ibid. 81*:1111–1114 (2004).

41. Dimmig, T., W. Radig, C. Knoll, and T. Dittmar, ^{13}C-NMR-Spektroskopie zur Bestimmung von Umsatz und Reaktionskinetik der Umesterung von Triglyceriden zu Methylestern [^{13}C-NMR Spectroscopic Determination of the Conversion and Reaction Kinetics of Transesterification of Triglycerols to Methyl Esters], *Chem. Tech. (Leipzig) 51*:326–329 (1999).
42. Knothe, G., Rapid Monitoring of Transesterification and Assessing Biodiesel Fuel Quality by NIR Spectroscopy Using a Fiber-Optic Probe, *J. Am. Oil Chem. Soc. 76*:795–800 (1999).
43. Knothe, G., Monitoring the Turnover of a Progressing Transesterification Reaction by Fiber-Optic NIR Spectroscopy with Correlation to ^{1}H NMR Spectroscopy, *Ibid. 77*:489–493 (2000).
44. Dubé, M.A., S. Zheng, D.D. McLean, and M. Kates, A Comparison of Attenuated Total Reflectance-FTIR Spectroscopy and GPC for Monitoring Biodiesel Production, *Ibid. 81*:599–603 (2004).
45. Siatis, N.G., A.C. Kimbaris, C.S. Pappas, P.A. Tarantilis, and M.G. Polissiou, Improvement of Biodiesel Production Based on the Application of Ultrasound: Monitoring of the Procedure by FTIR Spectroscopy, *Ibid. 83*:53–57 (2006).
46. Zagonel, G.F., P. Peralta-Zamora, and L.P. Ramos, Multivariate Monitoring of Soybean Oil Ethanolysis by FTIR, *Talanta 63*:1021–1025 (2004).
47. De Filippis, P., C. Giavarini, M. Scarsella, and M. Sorrentino, Transesterification Processes for Vegetable Oils: A Simple Control Method of Methyl Ester Content, *J. Am. Oil Chem. Soc. 72*:1399–1404 (1995).
48. Sadeghi-Jorabchi, H., V.M.E. Wood, F. Jeffery, A. Bruster-Davies, N. Loh, and D. Coombs, Estimation of Biodiesel in Lubricating Oil Using Fourier Transform Infrared Spectroscopy Combined with a Mid-Infrared Fibre Optic Probe, *Spectrosc. Europe 6*:16,18,20–21 (1994).
49. Siekmann, R.W., G.H. Pischinger, D. Blackman, and L.D. Carvalho, The Influence of Lubricant Contamination by Methyl Esters of Plant Oils on Oxidation Stability and Life, *Proc. Int. Conf. Plant and Vegetable Oils as Fuels*, published by ASAE, St. Joseph, Michigan, ASAE Publication 4-82, pp. 209–217 (1982).
50. Vähäoja, P., J. Närhi, T. Kuokkanen, O. Naatus, J. Jalonen, and S. Lahdelma, An Infrared Spectroscopic Method for Quantitative Analysis of Fatty Alcohols and Fatty Acid Esters in Machinery Oils, *Anal. Bioanal. Chem. 383*:305–311 (2005).
51. Bírová, A., E. Švajdlenka, J. Cvengroš, and V. Dostálíková, Determination of the Mass Fraction of Methyl Esters in Mixed Fuels, *Eur. J. Lipid Sci. Technol. 104*:271–277 (2002).
52. Pimentel, M.F., G.M.G.S. Ribeiro, R.S. da Cruz, L. Stragevitch, J.G.A. Pacheco Filho, and L.S.G. Teixeira, Determination of Biodiesel Content When Blended with Mineral Diesel Fuel Using Infrared Spectroscopy and Multivariate Calibration, *Microchem. J. 82*:201–206 (2006).
53. Knothe, G., Determining the Blend Level of Mixtures of Biodiesel with Conventional Diesel Fuel by Fiber-Optic NIR Spectroscopy and ^{1}H Nuclear Magnetic Resonance Spectroscopy, *J. Am. Oil Chem. Soc. 78*:1025–1028 (2001).
54. Foglia, T.A., K.C. Jones, and J.G. Phillips, Determination of Biodiesel and Triacylglycerols in Diesel Fuel by LC, *Chromatographia 62*:115–119 (2005).
55. Kami*f*ski, M., E. Gilgenast, A. Przyjazny, and G. Romanik, Procedure for and Results of Simultaneous Determination of Aromatic Hydrocarbons and Fatty Acid Methyl Esters in Diesel Fuels by High-Performance Liquid Chromatography, *J. Chromatogr. A 1122*:153–160 (2006).
56. Bondioli, P., A. Lanzani, E. Fedeli, M. Sala, and S. Veronese, Vegetable Oil Derivatives as Diesel Fuel Substitutes. Analytical Aspects. Note 4: Determination of Biodiesel and Diesel Fuel in Mixture, *Riv. Ital. Sostanze Grasse 71*:287–289 (1994).
57. Bondioli, P., and L. Della Bella, The Evaluation of Biodiesel Quality in Commercial Blends with Diesel Fuel, *Ibid. 80*:173–176 (2003).
58. Tat, M.E., and J.H. Van Gerpen, Biodiesel Blend Detection with a Fuel Composition Sensor, *Appl. Eng. Agricult. 19*:125–131 (2003).
59. Munack, A., J. Krahl, and H. Speckmann, A Fuel Sensor for Biodiesel, Fossil Diesel Fuel, and Their Blends, ASAE Paper No. 02-6081 (2002).
60. Munack, A., and J. Krahl, Erkennung des RME–Betriebs mittels eines Biodiesel-Kraftstoffsensors (Identifying Use of RME with a Biodiesel Fuel Sensor), *Landbauforsch. Völkenrode Sonderh.* (Special Issue) *257* (2003).

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