A Review of Chromatographic Characterization Techniques for Biodiesel and Biodiesel Blends

R.E. Pauls

Chemical Sciences and Engineering Division, Argonne National Laboratory, Argonne, IL 60439, USA

Abstract

This review surveys chromatographic technology that has been applied to the characterization of biodiesel and its blends. Typically, biodiesel consists of fatty acid methyl esters produced by transesterification of plant or animal derived triacylglycerols. Primary attention is given to the determination of trace impurities in biodiesel, such as methanol, glycerol, mono-, di-, and triacylglycerols, and sterol glucosides. The determination of the fatty acid methyl esters, trace impurities in biodiesel, and the determination of the biodiesel content of commercial blends of biodiesel in conventional diesel are also addressed.

Introduction

Biodiesel is produced by transesterification of vegetable or animal derived triacylglycerols with methanol. Currently, soy-, palm-, and rapeseed-derived oils are most frequently employed in biodiesel production. The resulting fatty acid methyl esters (FAME) are referred to as B100. Ethanol and other higher alcohols are also employed in the transesterification reaction in place of methanol. The pure FAME or B100 is blended with conventional diesel to produce commercial fuels. These fuels are referred to as Bx where x is the percent biodiesel or FAME blended; a 10 percent blend of B100 in conventional diesel is referred to as B10.

Both ASTM (1) and European Union (2) have published standard specifications for B100 FAME used in diesel blending. These specifications reference specific analytical methods including GC (methanol, free glycerol, mono-, di-, and triacylglycerols), elemental analysis, as well as physical property tests. Table I is adapted from the ASTM D 6751-08 specification for B100.

In addition to the standard tests cited in Table I, the chromatographic determination of a number of other biodiesel properties is of interest. These include determination of the amount of biodiesel in blends, identification and measurement of trace impurities in biodiesel that can negatively (or positively) impact biodiesel performance, and analyses performed to determine the type and origin of the FAME. The determination of the inadvertent contamination of other products such as jet fuel with biodiesel is also of increasing interest.

In this review we focus on the application of chromatographic methods for the characterization of biodiesel and its blends. Primary attention is given to methods related to final product analysis rather than those employed in monitoring transesterification reactions, although these are cited where appropriate. Many approaches employed to characterize B100 are similar to those employed in the food industry. In this review, a number of recent references from this area relevant to biodiesel are cited, especially those employing multidimensional separations. Many of these multidimensional approaches should see increased application to biodiesel in the future.

Table I. ASTM D 6751-08 Specification*

Property	Test Method	Limit	Units
Total Ca and Mg	EN 14538	5 max	ppm (µg/g)
Flash Point (closed cup)	D 93	93 min	°C
Alcohol Control			
Either:			
Methanol	EN 14110	0.2 max	% mass
Flash Point	D 93	130 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	% mass
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
Cold soak filterability	Annex A1 of D 6751	360 max	seconds
Free glycerol	D 6584	0.020 max	% mass
Total glycerol	D 6584	0.240 max	% mass
Phosphorus	D 4951	0.001 max	% mass
Distillation temperature,			
90% recovered	D 1160	360 max	°C
Total Na and K	EN 14538	5 max	ppm (µg/g)
Oxidation stability	EN 14112	3 min	hours

* Adapted with permission from ASTM D 6751 (1).

*Author to whom correspondence should be addressed: email repauls@sbcglobal.net

Reproduction (photocopying) of editorial content of this journal is prohibited without publisher's permission.

Outline

I. General References

Several reviews have recently surveyed the status of a wide range of analytical methods for the analysis and characterization of biodiesel (3–10). These reviews summarize the available analytical methods developed for both B100 and biodiesel blends as well as methods to monitor the extent of the transesterification reaction. The most recent of these (3) contains an assessment of a variety of spectroscopic and chromatographic methods suitable for biodiesel analyses.

II. Methanol

Methanol is the most common alcohol employed for the transesterification of triacylglycerides to produce biodiesel. This choice is driven primarily by methanol's low cost compared to that of other alcohols. However, in some areas of the world, such as Brazil, where ethanol is less expensive and readily available, it is used in place of methanol. Occasionally other higher alcohols have been employed for the production of biodiesel (11). The presence of residual methanol (or other alcohols) in biodiesel is undesirable and can lead to a low flashpoint.

The use of headspace gas chromatography (GC) to determine methanol in biodiesel is well established. Both ASTM and the European Union specify this approach. For example, EN 14110 (12) describes a headspace GC method suitable for the determination of trace methanol in B100 at concentrations ranging from 0.01 to 0.50 mass %. Typically, 2 mL of sample are placed in a 20 mL headspace vial, which is heated at 80°C for 45 min. An aliquot of the headspace (0.5 mL) is injected into a GC, and the methanol content is determined. Either internal (2-propanol) or external calibration is allowed. External standardization is only recommended with automated headspace equipment. A dimethyl siloxane capillary column with a film thickness exceeding 0.5 µm is suitable for this determination.

Despite its many attractive features, the major limitation of the headspace method is the need for automated headspace equipment and long sample preparation times. Several other approaches have been described to determine methanol in B100, although none appears to have achieved widespread application. In earlier work, Bondioli and co-workers (13) developed a method to determine methanol in biodiesel samples. A 50 g aliquot of sample was diluted with 75 mL water and 1 mL of a 10 % wt/wt citric acid solution, and the resulting solution was distilled. After distillation of 45 mL, ethanol internal standard was added, and the resulting solution was analyzed by GC on a packed column. Mittelbach et al. (14) employed a silylation GC approach to determine residual methanol and glycerol. The samples were silylated with BSTFA and analyzed on a 60 m DB-5 column. Ethanol was used as internal standard to determine the methanol content. Both mass spectrometric and FID detection were employed.

Paraschivescu and co-workers (15) described the use of headspace solid phase extraction (SPE) for the determination of methanol in biodiesel. They used carboxen–polydimethylsiloxane fibers in conjunction with an HP-5 capillary column. The average relative standard deviations and recoveries were reported to be 7% and 100%, respectively. Li and co-workers in a Chinese journal describe the use of multidimensional GC to determine methanol in B100 (9). After methanol and the internal standard, 1-propanol, elute onto the second column, the heavier components left on the precolumn are backflushed.

Arzamendi et al. (16) employed size exclusion chromatography (SEC) to monitor transesterification reaction products including methanol. A set of three columns with refractive index detection provided separation of mono-, di-, and triacylglycerols, FAME, glycerol and methanol. The latter was observed as a negative peak. This approach is more suitable for the determination of methanol in reaction mixtures than in final biodiesel product. Campo, Gonzalez, and Rodriguez described a simplified GC method to determine residual methanol in biodiesel (17). The sample is injected into a packed liner held at room temperature. Heavier components are trapped on the liner while methanol passes through onto a methyl silicone capillary column. Presumably frequent replacement of the liner and front of the column would be required with this approach.

Residual methanol has also been determined by a variation of an aqueous extraction technique previously developed for ethanol and other low molecular weight alcohols in gasoline (18,19). The biodiesel sample is diluted with iso-octane and extracted with water. The methanol content of the aqueous phase is then determined by GC on a thick film dimethyl siloxane capillary column.

III. Glycerol and Acylglycerols

Glycerol liberated by the transesterification reaction is typically removed from biodiesel product during processing by water washing. Trace levels of free, unextracted glycerol in B100 are undesirable because its presence can impact engine performance and emissions. The current ASTM specification for free glycerol is less than 0.020 mass %. Both the ASTM and European Union specifications also contain limitations on the amount of total glycerols given as the sum of free glycerol and bound glycerols (glycerol bound as mono-, di-, and triacylglycerols). The ASTM limit for total glycerol is less than 0.240 mass %.

ASTM/EN method

The most common approach to determine glycerol in biodiesel is silylation GC. ASTM D 6584 (20) describes a method

to determine the free and total glycerol content of B 100. The method is based upon high temperature silylation GC. Glycerol, mono-, di-, and triacylglycerols are detected by the method. The total glycerol content is obtained by summing the free glycerol content with the bound glycerol (i.e., the glycerol content of the mono-, di-, and triacylglycerols). The silylation reagent is *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA). A 10 m × 0.32 mm column coated with a 0.1 µm film of 5% phenylpolydimethylsiloxane is employed. Use of a 1–2 m precolumn is recommended. The column oven is temperature programmed up to 380°C via a multi-ramp program, and on-column injection is required. Two internal standards are employed: 1,2,4-butanetriol for the glycerol and 1,2,3-tridecanolylglycerol for the acylglycerols. Specific relative retention time windows are assigned to the mono-, di-, and triacylglycerols. Figure 1 contains a typical chromatogram of a soy-based B100.

Care must be used to correctly assign peaks, as there are potential overlaps. For instance, the C24:0 FAME elutes among the monoacylglycerols. A number of minor components also appear to elute in the vicinity of the triacylglycerols. The method is suitable for the detection of 0.005 wt% free glycerol and 0.05 wt% total glycerol. At the ASTM specification level of 0.24%, the repeatability and reproducibility for total glycerol are reported to be approximately 0.04 and 0.13 wt%, respectively. The corresponding EN silylation GC method for determining free and bound glycerol is EN 14105 (21). A large number of articles in the popular chromatography literature have discussed the implementation of D 6584/EN 14105. References 22–26 contain a limited sampling of these.

Methods for free glycerol

In addition to ASTM D6584 and EN 14105, several alternative methods have been reported for the determination of free glycerol. Bondioli and co-workers (27) developed a packed column GC-extraction method to determine residual glycerol in B100. After dilution with hexane and ethanol, the sample was extracted with an aqueous formic acid solution containing 1,4-butanediol as internal standard. The aqueous phase was then examined by GC. Interferences were noted for B100 derived from oils containing low molecular weight esters. In 1993, Mittelbach (28) reported a GC method to determine glycerol directly in FAME after reaction with bis(trimethylsilyl)-trifluoroacetamide (BSTFA) in dimethylformamide (DMF) solvent. Once again, 1,4-butanediol was used as internal standard, and the separation was performed on a 60 m DB-5 column.

Sala and Bondioli (29) compared two methods for the determination of residual glycerol. The first of these was a chemical method based on periodate oxidation of the glycerol to formaldehyde; the second was a high-performance liquid chromatography (HPLC) procedure. Comparable results were obtained with both methods, although each approach had its unique advantages. The chemical method required less sophisticated equipment; the HPLC method provided more detailed information on the sample. This group (30) also developed a chemical-spectrophotometric method that correlated well with GC methods.

Bansal and co-workers (31) discussed a thin-layer chromatography (TLC) method for the determination of glycerol in biodiesel. Detection limits were reported to be on the order of 0.1 to 0.2 vol%. Hajek et al. (32) reported an HPLC method to determine free glycerol in biodiesel. Their method was based on aqueous extraction followed by HPLC with refractive index detection. Results were reported to be comparable to those achieved by alternative GC methods.

Foglia and co-workers (33) discussed the determination of glycerol by normal phase HPLC. The sample was diluted in hexane and separated on a CN column under gradient elution conditions. An evaporative light scattering detector was employed. Detection limits on the order of 0.02 mass % appeared to be possible with this approach. Figure 2 shows chromatograms they obtained at high (1 wt%) and low (0.02 wt%) levels of glycerol with this approach.

Methods for bound glycerol

The EN and ASTM silylation GC methods for total glycerol were based on a number of earlier literature reports. For example, Freedman and co-workers (34) developed a fast capillary silylation GC method to monitor transesterification reactions. The column was a 1.8 m × 0.32 mm SE-30, and the

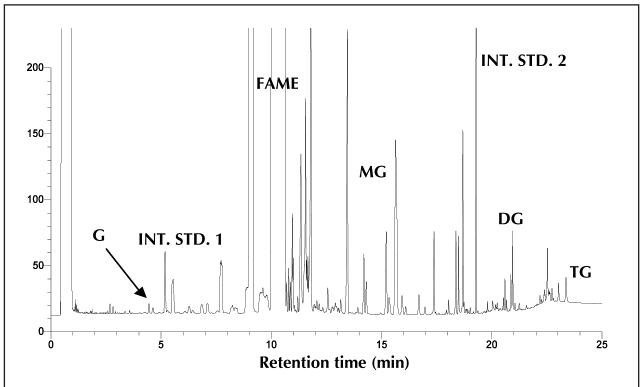

Figure 1. ASTM D 6584 chromatogram of soy-derived B100. Peak assignments: G = glycerol; Int. Std. 1 = 1,2,4 butanetriol; FAME = fatty acid methyl esters; MG = monoacylglycerols; Int. Std. 2 = 1,2,3-tridecanolylglycerol; DG = diacylglycerols; TG = triacylglycerols.

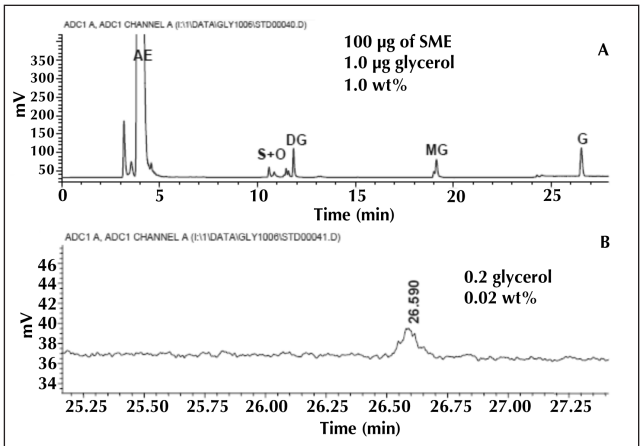

Figure 2. HPLC separation of biodiesel impurities. A: Chromatogram of high level standard containing 1 wt% glycerol. B: Expanded view of low level glycerol standard at 0.02 wt%. AE = FAME esters; S+O = sterols and oxidation products; DG = diacylglycerols; MG = monoacylglycerols; G = glycerol. Taken from reference 33.

column was temperature programmed to 350°C. Split injection was employed. BSTFA was employed as the silylation reagent. Analysis time was on the order of 12 min.

Mariani and co-workers (35) also described a similar silylation GC method to determine mono-, di-, and triacylglycerols as did Plank and Lorbeer of the University of Vienna (36,37). In Plank's work, MSTFA was used as the derivatization agent, and samples were separated on a 10 m × 0.32 mm DB-5 column with a 0.1 µm film. A 2 m pre-column was employed. Hydrogen was the carrier gas. The internal standards were the same as those later adopted by ASTM and the EN: 1,2,4-butanetriol and tricaprin. Recovery of spiked mono-, di-, and triacylglycerols was reported to be acceptable, except for low levels of triacylglycerols. The relative standard deviations obtained for a set of seven replicates of a rapeseed methyl ester sample were on the order of 0.5 to 0.9%. Wawrzyniak and Wasiak (38) have also recently discussed the determination of residual acylglycerols directly in diesel oil by silylation GC with MSTFA derivatitzation.

Lechner, Bauer-Plank, and Lorbeer (39) subsequently developed an on-line LC-GC method to determine mono-, di-, and triacylglycerols in B100. The sample was initially derivatized by acetylation of the hydroxyl groups on the mono- and diacylglycerols and these were then separated by normal phase HPLC. A loop-type interface with concurrent eluent evaporation was used to transfer HPLC fractions onto the GC column.

Fialkov, Gordin, and Amirav (40) have explored the use of a supersonic mass spectrometer coupled to a GC for the characterization of a range of fuels including B100. A short 4 m column with temperature programming up to 360°C was employed in this work. The primary advantage of the supersonic mass spectrometer over other mass spectrometric approaches was that it provided almost exclusively molecular ions.

The silylation GC methods described earlier suffer from a number of limitations, including the need for derivatization and high GC column temperatures as well as the poor resolution of some acylglycerols. To address this issue, a number of HPLC methods, both normal and reversed phase, were developed to determine acylglycerols in B100. However, to date, these methods have not received wide acceptance.

Foglia and co-workers at the USDA evaluated different chromatographic methods to determine the bound glycerol in B100 (33,41,42). These included the high-temperature silylation GC method described in ASTM D 6584 as well as a normal phase HPLC method. The LC method employed a CN column (250 × 4.6 mm, 5 µm particles), and the mobile phase components were hexane (A) and methyl tertiary butyl ether (B), each containing 0.4% acetic acid. The mobile phase was linearly programmed from 0% B to 80% B over 10 min following an initial 5 min hold. An evaporative light scattering detector was employed. One advantage of the HPLC method was that it could quantify free acids such as oleic acid.

Analyses were performed on samples of soy and rapeseed FAME. These materials were initially purified by column chromatography to remove traces of acylglycerols. Known amounts of acylglycerols were then blended back into the samples. For most spiked samples, no statistical differences were observed between results obtained with the HPLC and ASTM 6584 methods. However, differences were noted between the expected and found diacylglycerol content in some soy samples by the HPLC method. Subsequent LCMS experiments suggested this was due to the co-elution of oxidized FAME components with the diacylglycerols.

Holcapek et al. (43) employed non-aqueous reversed phase HPLC with UV (205 nm), evaporative light scattering (ELSD) and APCI mass spectrometric detection to characterize products of transesterification reactions. A detailed discussion of the relative performance of each detection method was provided. A dual step, ternary gradient was found to give good separations of free acids, methyl esters, and mono-, di-, and triacylglycerols. The three mobile phase components were (A = water; B = acetonitrile; C = isopropanol–hexane, 5/4 v/v). The gradient was 30% A–70% B initial; 100% B in 10 min; then 50% B–50% C at 20 min, followed by a 5 min hold. This also provided separation of positional isomers of the mono- and diacylglycerols (i.e. 1,2- vs. 1,3-substituted diacylglycerols). Positive ion APCI ionization was employed in the LC–MS studies and was found to provide low limits of detection as well as providing structural information. This group has also explored in detail the characterization of mono-, di-, and triacylglycerols in a variety of seed oils by HPLC with multiple detection systems (44-47).

Di Nicola and co-workers (48) developed a reversed-phase HPLC method with UV detection to analyze biodiesel mixtures. A rigorous statistical design based on full factorial design and steepest ascent optimization was employed to select chromatographic parameters. Detection was at 210 nm. The elution order was free acids, followed by monoacylglycerols, FAME, diacylglycerols, and triacylglycerols. Tuerkan and Kalay (49) developed a reversed-phase HPLC method to separate products from the lipase-catalyzed methanolysis of sunflower oil. LC–MS studies with APCI were also performed. An ion trap analyzer was utilized. Santori and co-workers (50) described a reversed-phase HPLC method to determine the composition of transesterification reactions. UV detection was employed. Detector response was found to vary with the number of double bonds in the FAME. Figure 3 contains an HPLC chromatogram of the upper layer from a sunflower transesterification reaction from this report.

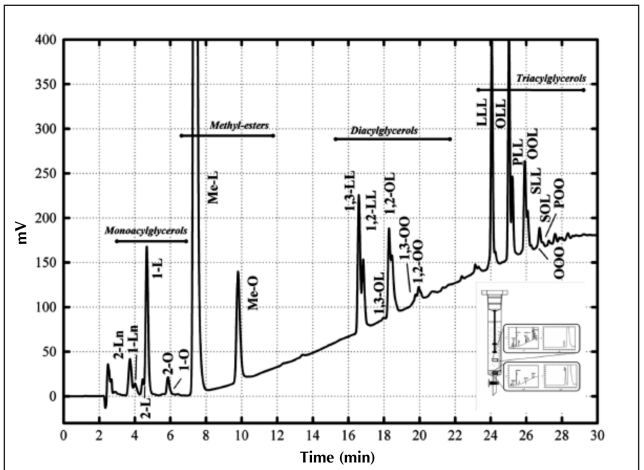

Figure 3. HPLC chromatogram of upper layer from a sunflower transesterification reaction. Reversed phase separation with UV detection. Taken from reference 50.

The use of ion-exchange chromatography coupled to pulsed amperometric detection has also recently been described and is under evaluation by ASTM for the determination of free and total glycerol (51,52). Free glycerol is determined after aqueous extraction of the biodiesel sample. Total glycerol is determined after saponification and extraction.

SEC has been employed by a number of research groups to separate biodiesel components. This technique is especially useful to monitor the extent of transesterification reactions. Trathnigg and Mittelbach (53) used a coupled system of a CN normal phase column connected to 100 Å and 500 Å Styragel columns. The mobile phase was 0.6% ethanol in chloroform. A density detector was employed. The elution order was triacylglycerols followed by diacylglycerols, FAMEs, free acids, and monoacylglycerols. Fillieres and co-workers (54) used SEC to determine the products from the ethanolysis of triacylglycerols.

Darnoko, Cheryan, and Perkins (55) described the separation of transesterification products on two coupled Phenogel columns with refractive index detection and THF mobile phase. Dube and co-workers (56) based their method on that of Darnoko with some modifications and compared results from GPC with those obtained by FTIR. Limitations of each approach were cited. Arzamendi and co-workers (16) used SEC to determine mono-, di-, and triacylglycerols, FAMEs, glycerol, and methanol. A variety of column configurations were explored. The final set consisted of three Styragel columns in series. Kittirattanapiboon and Krisnangkura (57) optimized the mobile phase composition to enhance the size exclusion chromatographic separation of mono-, di-, and triacylglycerols, and FAME.

Despite the disadvantages of the silylation GC method, HPLC methods have yet to have an impact on routine analyses of biodiesel. This appears to be primarily due to detector limitations. LC–MS is costly and complex for routine biodiesel applications, and both UV and ESLD detectors suffer from variations in response with molecular structure. However, with further advancements in detector technology, HPLC methods may begin to compete with GC approaches in the future.

In miscellaneous reports, Freedman and co-workers (58) had described the use of TLC with flame ionization detection to determine mono-, di-, and triacylglycerols in B100, and Fontana and co-workers (59) reported a screening method for the determination of mono-, di-, and triacylglycerols by TLC. Results were found comparable to those obtained by GC. Cole and co-workers (60) briefly discussed the use of supercritical fluid chromatography to separate free acids, FAME, and glycerol in B100.

IV. FAME Distribution

GC Methods

The literature on GC separations of FAMEs is extensive (61), and a comprehensive discussion of FAME analysis by GC is beyond the scope of this work. The separation of FAME mixtures by capillary GC is typically performed on polar phases, most commonly polyethylene glycol (PEG). More recently biscyanopropyl polysiloxane based phases have been employed to enhance the separation of cis-trans isomers (62–64). The two columns show slightly different selectivities. For example, the C20:0 FAME elutes prior to the C18:3 on the biscyanopropyl polysiloxane based phase. Figure 4 contains chromatograms of a soy-based and a beef tallow B100 on a PEG phase. Note the more complex distribution of FAME in the beef tallow compared to the soy. Figure 5 contains a chromatogram of soy FAME on a biscyanopropyl phase. Comparison with Figure 4A illustrates the differences in selectivity for soy FAME components on this and the PEG column. Also note the differences in analysis time.

Among more recent studies of GC FAME separations are those of Yamamoto and co-workers (65) who discussed separation of FAME on weakly polar columns and of Poole and co-workers (66) who used a solvation parameter model to characterize a number of stationary phases for their use in separating FAME. Harynuk and co-workers (67) have also examined new stationary phases for the separation of FAME. Ruiz-Rodriguez and co-workers (68) have recently reviewed advances in the analysis of fatty acids.

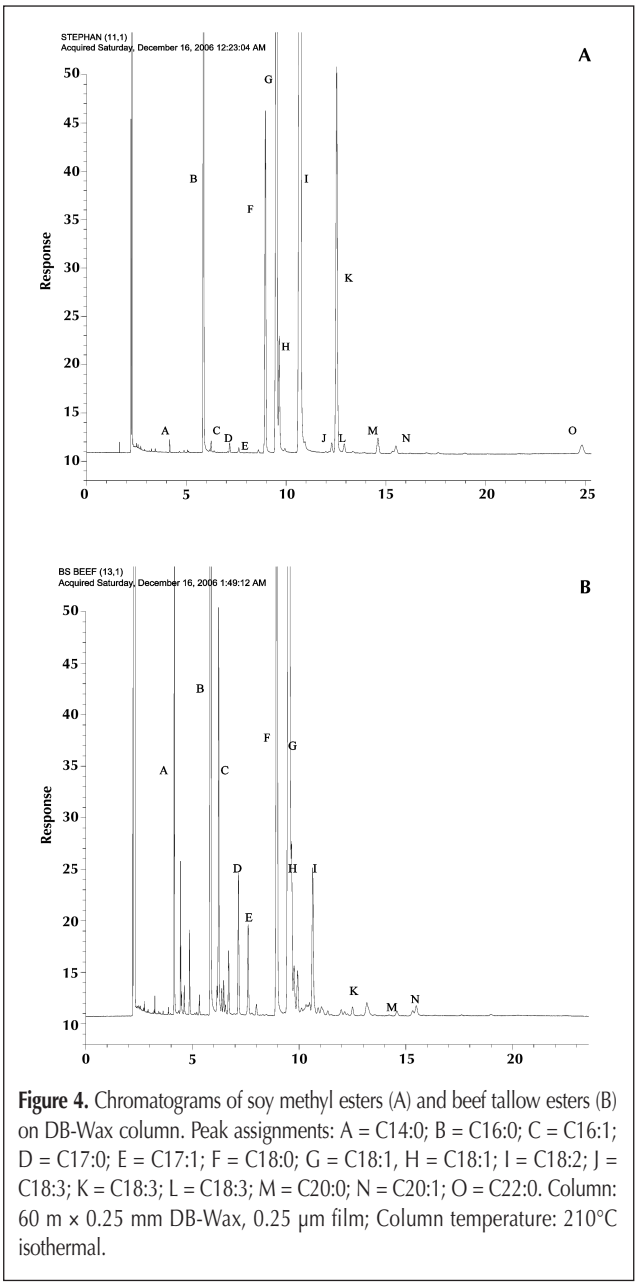

Figure 4. Chromatograms of soy methyl esters (A) and beef tallow esters (B) on DB-Wax column. Peak assignments: A = C14:0; B = C16:0; C = C16:1; D = C17:0; E = C17:1; F = C18:0; G = C18:1, H = C18:1; I = C18:2; J = C18:3; K = C18:3; L = C18:3; M = C20:0; N = C20:1; O = C22:0. Column: 60 m × 0.25 mm DB-Wax, 0.25 µm film; Column temperature: 210°C isothermal.

EN 14103 (69) describes a GC method to determine the total ester and the linolenic methyl ester content of B100. The method allows verification that the B100 meets European specifications for ester content and contains acceptable levels of linolenic acid FAME. B100 mixtures containing C14 through C24 FAMEs can be analyzed by this method. Samples are prepared by dissolving 0.25 g of FAME in 5 mL of heptane containing 10 mg/mL methyl heptadecanoate. The ester content is calculated from the area of all peaks, excluding the internal standard, between the C14 and C24 methyl esters. The linolenic acid FAME content is taken as the sum of three C18:3 peaks, one major and two minor. The repeatability and reproducibility of the linolenic acid FAME determination are reported to be 0.1 wt% and 0.3 × the concentration of linolenic acid plus 0.02 wt%, respectively. The recommended conditions call for a 30 m polyethylene glycol column (0.32 mm i.d., 0.25-µm film thickness), split injection, and isothermal separation at 200°C. This approach is not suitable for FAME mixtures that contain methyl heptadecanoate, such as some animal derived materials, as this method employs it as internal standard. Mittelbach and co-workers (70) have evaluated a modification of EN 14103 suitable for use on animal derived materials that uses an alternative internal standard. A number of workers in the Chinese literature have applied chromatographic techniques including GC–MS and HPLC to determine FAME distributions in biodiesel (71–75).

Recently a number of workers have reported the use of high speed GC to determine FAME distributions (76–81). Frequently these analyses employ narrow diameter capillary columns. Figure 6 contains a chromatogram of a palm-derived FAME on a 20 m × 0.100 mm PEG column. The column temperature was maintained at 240°C with a column head pressure of 90 psi (helium carrier gas). Analysis time was on the order of 4 min (82).

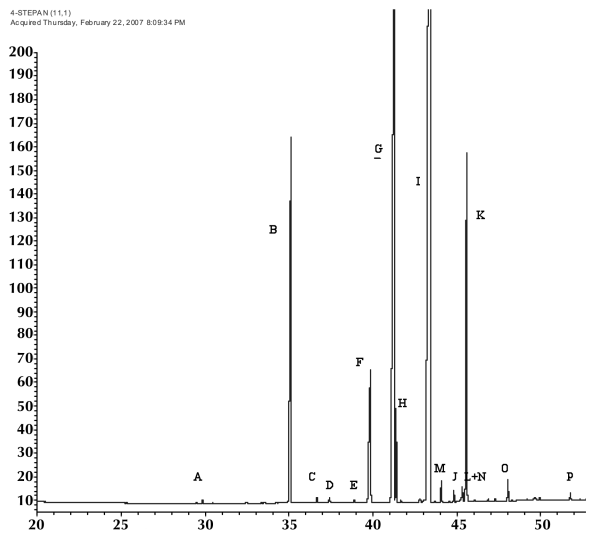

Figure 5. Chromatogram of soy methyl esters on Rtx 2560 column. Peak assignments: A = C14:0; B = C16:0; C = C16:1; D = C17:0; E = C17:1; F = C18:0; G = C18:1, H = C18:1; I = C18:2; J = C18:3; K = C18:3; L = C18:3; M = C20:0; N = C20:1; O = C22:0; P = C24:0. Column: 100 m x 0.25 mm Rtx 2560, 0.20 µm film; column temperature: 100°C for 4 min then 3°C/min to 240°C, hold 10 min.

Multidimensional separations

Since the first report of comprehensive 2D GC by Philips (83), this technology has been widely applied to a range of problems including FAME analysis. This has been accompanied by development of a number of other comprehensive multidimensional techniques. Recently, multidimensional approaches to chromatographic separations including food applications have been discussed (84). Mondello and co-workers reviewed the application of comprehensive chromatographic techniques to lipids such as FAME (85). Tiyapongpattana, Wilairat, and Marriott (86) characterized B100 using comprehensive two-dimensional GC. A combination BP5/BP20 (nonpolar/polar) column set was employed. A novel feature of this work was the use of a temperature programmed cryogenic modulator. The procedure was also suitable for biodiesel blends. Figure 7 shows a two dimensional GC chromatogram of a mixture of 37 FAME components. A number of other workers have examined comprehensive two-dimensional GC for the separation of FAME mixtures. References 87–92 contain representative examples.

Brinkman and co-workers (93) discussed the use of comprehensive LC × GC both with and without TOF mass spectrometry for FAME characterization. Increasingly, comprehensive two-dimensional LC is being used in the analysis of complex mixtures, especially for triacylglycerols (94–96). Francois and Sandra recently discussed comprehensive SFC × LC for the analysis of fatty acids (97), and Hirata and Sogabe (98) discussed SFC × SFC for ester characterization.

Hejazi and co-workers (99) evaluated the use of GC × field ionization mass spectrometry (FIMS) rather than GC × GC as a comprehensive two-dimensional separation method for the characterization of FAME mixtures (100,101). A time-of-flight mass spectrometer equipped with a field ionization source was employed; under field ionization conditions, the spectra consisted primarily of molecular ions. Separations were performed on a 60 m BPX-70 column. Data were displayed in a two-dimensional format with molecular mass as the x-axis and an adjusted retention time (relative to the saturated FAMEs) as the y-axis. The method was applied to a number of FAME mixtures including B100.

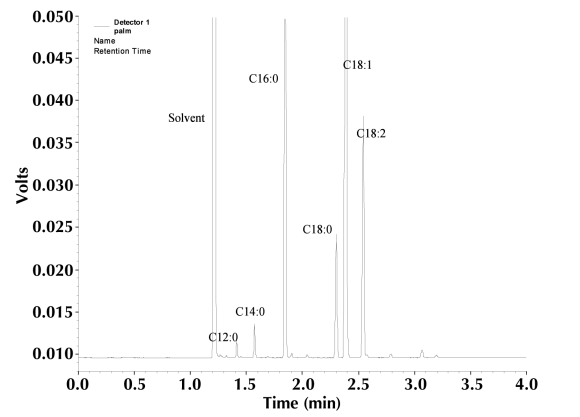

Figure 6. Chromatogram of palm methyl esters on a 100 µm capillary column. Column: 20 m x 0.10 mm DB-Wax with a 0.10 µm film. Column temperature: 240°C isothermal; 0. 2 µL injection; 500:1 split ratio.

Hartig (102) obtained retention times and mass spectra on more than 130 FAME with carbon numbers ranging from 4 to 24 carbons. Retention times on multiple columns were obtained that enabled more reliable identification of FAME.

LC and SFC

A number of LC techniques have also been employed to characterize FAME mixtures (103). In particular, silver ion complexation has been used to enhance the separation of unsaturated FAME (104,105). Gaudin and co-workers (106) recently examined the use of graphitic carbon for the separation of FAME. Several workers have also employed supercritical fluid chromatography to characterize FAME mixtures (107,108).

V. Determination of the B100 Content of Diesel Blends

FTIR and other spectroscopic methods are becoming the preferred approach to routinely and rapidly determine the amount of B100 found in biodiesel blends. For example, EN 14078 (109) describes an infrared method based on detection at a single wavelength, 1746 cm^{-1}, and a number of simple instruments have been marketed for this application. Increasingly, these spectroscopic methods are incorporating chemometric modeling to deal with blends from multiple sources of B100. References 110–114 contain representative examples of FTIR, Raman and NMR methods. Reference 3 contains a more detailed compilation of references on this topic.

Despite the speed and simplicity of spectroscopic approaches, chromatographic methods offer unique advantages. These determine not only the amount of B100 but also provide information on the source of the B100. These chromatographic methods frequently incorporate SPE to prefractionate FAME from the bulk diesel hydrocarbons. For example, Bondioli and co-workers (115) described the use of silica SPE cartridges to isolate biodiesel from diesel. The isolated material was then silylated and examined as described in reference 35.

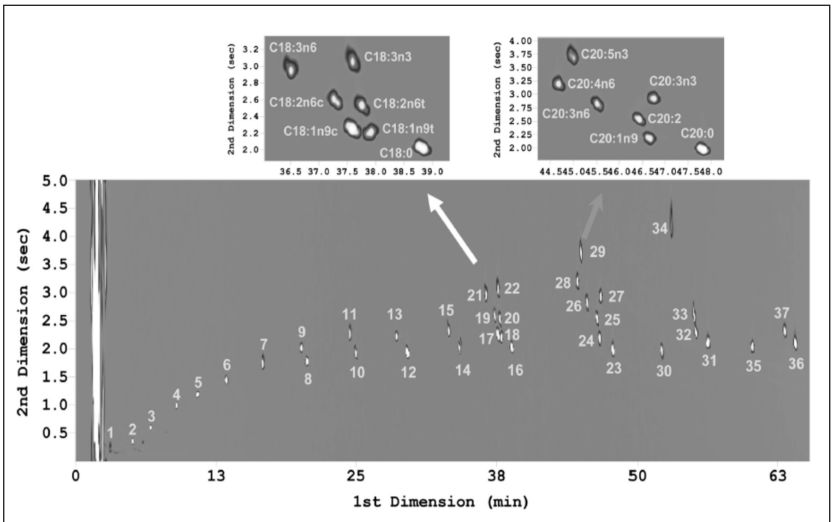

Figure 7. Two dimensional GC chromatogram of 37 FAME components. Taken from reference 86.

The EN chromatographic method for determining biodiesel in middles distillates is EN 14331 (116). This method provides a distribution of FAME components in the biodiesel, although it could be readily extended to include determination of the absolute amount by the addition of an internal standard. In this method, the sample is loaded onto a silica SPE cartridge followed by elution with hexane to remove the diesel fuel. FAME components are eluted with diethyl ether. This fraction is then separated on a PEG capillary column.

Wawrzyniak and co-workers (117) directly separated FAME components in biodiesel–diesel blends without pre-separation by GC with FID detection. They used an Innowax column with temperature programming.

Adam and co-workers (118) employed comprehensive two-dimensional GC with TOF-MS detection to determine both the FAME and hydrocarbon components in biodiesel blends. In initial evaluations of the separations, poor resolution was obtained between FAME and diesel components using a standard non-polar-polar column combination. This led to an investigation of various column combinations. As a result of these studies, a PEG column, 30 m × 0.25 mm × 0. 25 µm film (Solgel Wax), was selected for the first dimension column and a DB-1, 1 m × 0.1 mm, 0.1 µm film, was selected as the second dimension column. With this combination, FAME eluted in the vicinity of the mono-aromatics. However, above C14 FAME there were no significant overlap problems. Using this approach, hydrocarbon group type analyses of FAME-containing diesels (alkanes, naphthenes, mono-aromatics, di-aromatics, and tri-aromatics as well as the FAME content) were obtained. Good agreement for the biodiesel content was achieved between this approach and standard reference methods.

Seeley and co-workers (119,120) also employed comprehensive two-dimensional GC to determine the FAME composition of 1–20% diesel blends. The primary column was a 5% phenyl column while a PEG served as the secondary column. McCurry and Wang (121) discussed the use of Dean's switching for the analysis of FAME in biodiesel blends.

Tang and co-workers (122) used a GC–MS method to determine the properties of biodiesel blends marketed at 24 retail stations. This included the B100 content. They employed a GC–MS method with separation on a 60 m PEG column. FAME components with carbon numbers from 12 to 24 were detected. The total ion count of each FAME component was used for quantitation.

In addition to GC approaches, several authors have applied HPLC and SFC approaches to determine the amount of B100 in biodiesel blends. For example, Foglia and co-workers (33,123) reported a rapid HPLC method to determine the triacylglycerol and FAME content of biodiesel blends. Blends containing 1 to 30% biodiesel were examined. Both UV (201 nm) and evaporative light scattering detectors (ELSD) were evaluated. The column was a 200 × 4.6 mm Hypersil silica column packed with 5 µm particles. The mobile phase was 10% MTBE (methyl tertiary butyl ether) in hexane at a flow rate of 1 mL/min.

Analysis times were 5 min. For soy-derived materials, the UV and ELSD detectors gave comparable results. However, the use of the ELSD detector was preferred because its response was more uniform for all FAME components and could be more readily used for B100's from different sources. In contrast, the response of the UV detector was a function of the amount of unsaturated FAME.

Kaminski and co-workers (124) developed a method to determine aromatic hydrocarbons (by ring number) and FAME in diesel blends by HPLC. A LiChromspher NH_2 column was used to achieve the separation. Quantitative analysis was by refractive index although a diode array detector was placed in series to aid in cut point identification. FAME components were backflushed from the column after elution of hydrocarbons and determined as a group.

Diehl and DiSanzo (125) modified ASTM D 5186, an SFC method for aromatics in diesel, to allow determination of B100 in biodiesel. The modification consisted of addition of a backflush valve. After a normal D 5186 analysis, the silica gel column is backflushed eluting the strongly retained FAME. The average relative error in the analysis was approximately 2%. White (126) has recently discussed the use of SFC for biodiesel analysis.

VI. Determination of FAME in Jet Fuel

Contamination of jet fuel with B100 in common carrier pipelines is a growing concern for the aviation industry especially in Europe. Concentrations of FAME in jet as low as 5 ppm are of concern. This led to an investigation into possible methods for the determination of FAME. The most promising to date is based on GC–MS with selected ion detection (127). This method employs a 50 m PEG capillary column, and six specific FAME components are determined. Figure 8 shows selected ion chromatogram of the C16:0 FAME under these conditions. Concentrations down to 0.5 mg/kg could be readily detected. McCurry and co-workers (128,129) have recently reported on multidimensional GC approaches to determine trace B100 in jet fuel at trace concentrations. Hartman and co-workers (130,131) have recently reported a SPE-FTIR method to rapidly screen aviation turbine fuels for the presence of FAME, and Read and Hooks have discussed alternate analytical methods currently under development for trace levels of FAME in aviation turbine fuels (132).

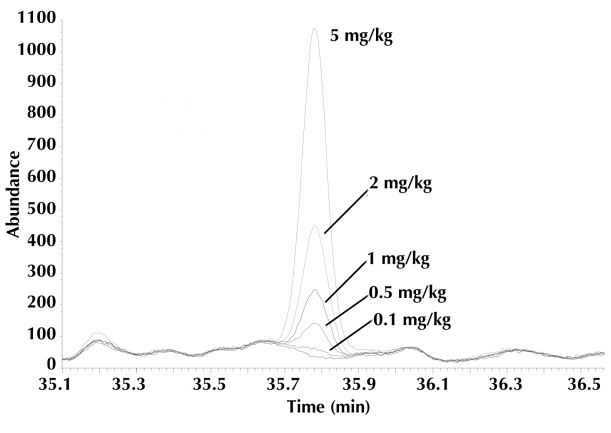

Figure 8. Selected ion chromatograms of C16:0 FAME at varying concentrations in jet fuel. From reference 127.

VII. Trace Biodiesel Impurities in B100 including Sterols and Tocopherols

After transesterification, a number of trace impurities in addition to residual mono-, di-, and triacylglycerols are found in B100. Among the more prominent are free sterols, tocopherols and sterol glucosides (discussed separately below). Figure 9 contains an expanded view of a D 6584 chromatogram of a soy FAME showing the location of these components. The distribution of sterols and tocopherols differs among various seed oils and, as discussed later, this may be useful in identifying the source of B100. The sterols as well as the tocopherols can be removed by distillation of the FAME product and their absence may be an indicator the B100 has been distilled or otherwise treated. The impact of these and other impurities on biodiesel quality and stability is poorly understood at this time. Some of these components, such as tocopherols, may positively impact biodiesel quality by acting as natural antioxidants. Many of these trace impurities can be determined by a silylation GC methods similar to that described in ASTM D 6584 and elsewhere (20,133).

The distribution of free sterols in soy and rapeseed differ. Both contain campesterol and β-sitosterol as major components. However, soy contains stigmasterol as the third main sterol while the third main sterol in rapeseed is brassicasterol. In soy, the relative ratio of campesterol–stigmasterol–β-sitosterol is reported to be 1:1:2.5 (134)

The determination of sterol composition is typically performed by GC analysis after silylation. A recent example describing this approach is by Bowden and co-workers (135) who investigated the use of microwave assisted silylation to derivatize steroids.

Plank and Lorbeer described a GC method to characterize the sterol and sterol ester constituents of rapeseed FAME (136). Prior

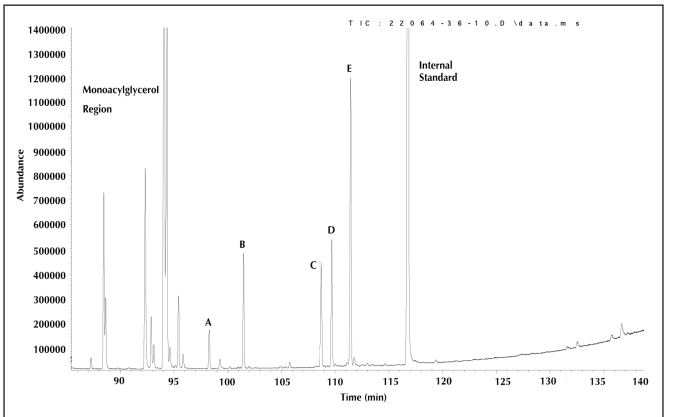

Figure 9. Expanded view of D 6584 chromatogram of soy FAME showing major tocopherol and sterol components. A = delta tocopherol; B= gamma tocopherol; C = campesterol; D= stigmasterol; E = beta sitosterol.

to analysis, samples were silylated. Betulinol was added as internal standard. The column was a 10 m × 0.32 mm DB-5 with a 0.1 µm film. On-column injection was employed. The column oven was programmed in three stages up to 350°C. Six free sterols were observed (β-sitosterol, campesterol, brassicasterol, cholesterol, stigmasterol, and Δ^5-avenasterol, in order of decreasing concentration). Total free sterol concentrations in six rapeseed methyl esters ranged from 0.34 to 0.50 wt%. On average, β-sitosterol accounted for 47% of the total followed by campesterol (34%) and brassicasterol (13%). The sterol esters were not completely separated from each other and were reported as a group. The two largest peaks were assigned as the C18 esters of β-sitosterol and campesterol. The C18 fatty acid was not specified. The total ester content for the same six samples ranged from 0.59 to 0.77 wt%. These authors further described the application of combined LC–GC (137,138) to determine free and esterified sterols in B100. Optimization of the transfer properties of eluent between the LC and GC was performed. Good recoveries of spiked components were achieved

The HPLC procedures developed by Foglia (33,41) may also be useful for the determination of sterols, sterol esters, and other impurities in B100. B100's also contain low levels of oxidized and other polar FAME's (41). The impact of these trace contaminants on product quality has not been evaluated to our knowledge.

Tocopherols are a family of naturally occurring phenolic antioxidants. Three different tocopherols, alpha, delta, and gamma are the major species typically found in seed oils such as rapeseed and soy. These compounds differ in the number of methyl groups located on the tocopherol aromatic ring. In soy, the delta and gamma forms predominate while rapeseed, the gamma and alpha are the predominate forms.

A number of HPLC methods are available for the determination of tocopherols in vegetable derived oils. For example, Schwartz and co-workers (139) used normal-phase HPLC with fluorescence detection while Gruszka and Kruk (140) used reversed-phase HPLC also with fluorescent detection. Pauls and co-workers (82) described a UPLC method to determine tocopherols in B 100 with UV detection. Analysis time was on the order of 3–4 min. Bostyn and co-workers used HPLC to study the degradation of tocopherols in rapeseed FAME (141), while Frohlich and Schober have studied the effect of tocopherols on the oxidative stability of FAMEs (142).

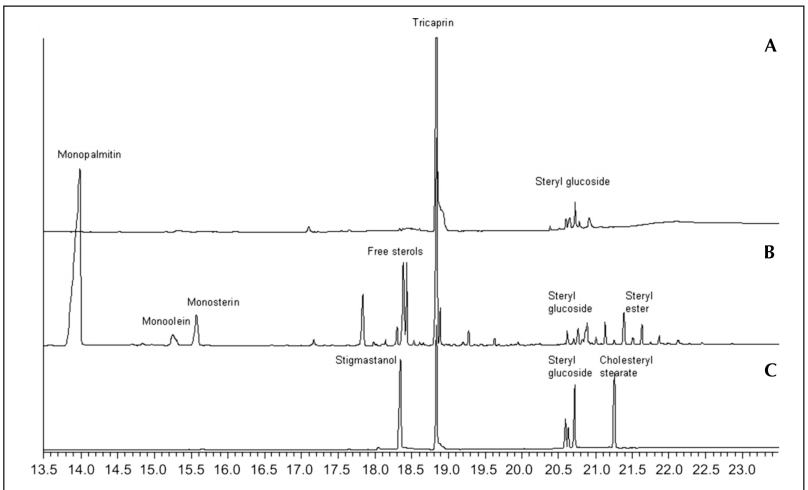

Figure 10. Representative GC-FID chromatograms of precipitates from soybean oil-based biodiesel (A); cotton seed oil based biodiesel (B); and reference mixtures of sterol glucoside, stigmasterol, and cholesteryl stearate (C). Taken from reference 150.

VIII. Sterol Glucosides

Sterol gluocosides consist of a sugar unit glycosidically linked to a sterol. Sterol glucosides are initially present in vegetable oils in an acylated form with a long chain fatty acid esterified to sugar. However during the transesterification reaction, this fatty acid side chain is removed leaving the sterol glucoside. Once the fatty acid group is removed, the solubility of these components is drastically reduced. Sterol glucosides have been reported to be responsible for solids formation in biodiesel after it has been subjected to low temperatures (143–151). These solids also typically contain co-precipitated monoglycerides containing only saturated fatty acids as well as other trace impurities (146).

Early work on method development for determining sterol glucosides included that of Byrne and co-workers (152) who developed an HPLC method for sterol glucosides at trace levels in whiskey, and that of Knights (153) who discussed the derivatization and GC–MS of these compounds. The mass spectra of trifluoroacetate, heptafluorobuyrate, and trimethy silyl derivatives were presented. Phillips et al. (154) discussed a method to determine sterol glucosides in food using SPE and silylation GC. More recently, Pfalzgraf and co-workers (143) described a GC method to determine these components in B100. This involved silylation followed by separation on a DB-5HT column (30 m × 0.32 mm with a 0.1 µm film). A three stage temperature program was employed. They reported sterol glucoside concentrations of 120 ppm for palm, 60 ppm for soy, and 20 ppm for canola derived B100.

Van Hoed and co-workers (147) concentrated heavier impurities in palm and soy-derived biodiesel and examined the heavy components by GC after silylation with BSTFA. A multistage temperature program was employed with a final temperature of 360°C. Mass and NMR spectra of underivatized sterol glucosides were also obtained. Concentrations of sterol glucosides in palm biodiesel ranged from 55 to 275 mg/kg while it ranged from non-detectable to 158 mg/kg for soy biodiesel.

Bondioli, Cortesi, and Mariani (148) developed a method to determine sterol glucosides in palm and soy-derived B100. They initially concentrated the sterol glucosides by SPE on silica. The residue was then examined by silylation (BSTFA) GC with and without a methanolysis step to convert the glucosides back to their corresponding free sterols. It was necessary to control the severity of the methanolysis step to minimize dehydration of the sterols. They also confirmed the identification of biodiesel precipitants as sterol glucosides. A BD-5HT column (10 m × 0.32 mm, 0.1 µm film thickness) with a multistep temperature program was employed. The final temperature was 370°C.

Moreau and co-workers (149) also investigated methods to confirm the presence of sterol glucosides in biodiesel precipitants. Both normal and reversed-phase HPLC methods were employed with UV, ELSD, and mass spectrometric detection. A quantitative method to determine sterol gluocosides in precipitants was developed based on isocratic normal phase HPLC with ELSD detection. Ng and co-workers (150,151) have studied the formation of precipitants in biodiesel derived from a number of sources. They used silylation (MSTFA) GC with FID detection to characterize these precipitants. Both sterol glucosides and monoglycerides were identified in precipitants. Figure 10 contains representative GC–FID chromatograms of precipitates from soybean oil-based biodiesel; cotton seed oil-based biodiesel and reference mixtures of sterol glucoside, stigmasterol and cholesteryl stearate. Lacoste and co-workers (155) have also developed a method based on HPLC isolation of steryl glucosides followed by silylation GC.

Shea and Pauls (156) have studied the mass spectrum of β-sitosterol glucoside and other rapeseed-derived glucosides after silylation. The molecular weight of the silylated β-sitosterol glucoside is 864. The primary fragments are observed at mass 204 (a typical fragment in the spectrum of silylated glucose) and at mass 397 due to fragmentation at the ether linkage.

IX. Miscellaneous

In addition to the many LC–MS approaches cited earlier, a number of direct mass spectroscopic methods have been reported for the characterization of B100. Many of these involve use of direct infusion mass spectrometry with LC–MS type interfaces (i.e., electrospray or APCI). Eide and Zahlsen (157) discussed the use of electrospray mass spectrometry in conjunction with chemometrics to identify the source of the B100, to identify the individual FAMEs present and to quantify the amount of B100 in blends. Samples were dissolved in dichloromethane and directly injected into an acetonitrile–ammonium acetate mobile phase that flowed into the electrospray source. A quadrupole mass spectrometer was employed. Positive ions were monitored to detect FAMEs and negative ions were monitored to detect free acids. The FAME was detected as ammonium adducts.

Catharino and co-workers (158) also used direct infusion electrospray mass spectrometry to fingerprint B100. A Q-TOF spectrometer was employed and both positive and negative ions were examined. Abdelnur and co-workers (159) employed desorption-ionization mass spectrometry to examine B100 in order to identify the source of the biodiesel. A drop of sample was placed on a sheet of paper and analyzed at ambient conditions. Both positive and negative ions were examined. Cooks and co-workers (160) have recently explored the use of reactive desorption electrospray mass spectrometry to determine cholesterol in a variety of matrices. Such an approach may provide a rapid technique to determine trace impurities in biodiesel.

Identification of the source of biodiesel (i.e., soy, rapeseed, palm, animal fat, etc.) is often of interest to biodiesel customers. A considerable literature exists in the fats and oils industry on the identification of the source of oils and on issues related to adulteration. A few examples are provided here. Aparicio and Aparicio-Ruiz (161) reviewed the use of chromatographic techniques to identify adulteration of vegetable oils. Although their focus was primarily on the adulteration of olive oil, many of the concepts they discussed are applicable to identifying the source and possible contamination of B 100. Reported tools for detecting adulteration include determining the distribution of FAME components, the composition of triglycerides and the distribution of sterols and tocopherols. The latter topic was briefly addressed above in regard to soy and rapeseed B100.

Fan and co-workers (162) determined the FAME distribution of biodiesel blends by GC and used this information to determine the source of the B100. A plot of the ratio of C16:0 to C18:0 vs. the ratio of C18:2 to C18:1 was useful for this purpose. Ruiz-Mendez and co-workers (163) used a combination of adsorption and size exclusion liquid chromatography to characterize FAME derived from frying oils. The frying process induces a number of changes in the oil leading to the formation of oligomeric and polar FAME. This approach may be useful for detecting the presence of frying oils in B100.

Giordani and co-workers (164) employed electronic nose technology to identify the source of B100. The chemical sensor array had 32 elements and principal components analysis was used to classify B100 and biodiesel blends. Headspace sampling was employed.

Another concern related to the introduction of B100 into diesel blends is its impact on standard specification tests for diesel fuels. For example, the ASTM diesel specification now allows for the presence of B100 (165). Standard ASTM tests used for diesel include D 2887, simulated distillation to determine the boiling point distribution of diesel, D 5186, determination of aromatics in diesel, and ASTM D 6591 determination of the ring number distribution of middle distillates (166–168). These will need to be modified to account for the presence of B100. The Energy Institute in the UK is working in conjunction with ASTM to address many of these issues (169). Mittlebach and co-workers (170) have recently explored the use of simulated distillation to characterize various types of biodiesel.

Summary

A wide variety of chromatographic technologies have been successfully applied to characterize biodiesel, trace impurities in biodiesel and the composition of biodiesel-diesel blends. Many of these methodologies employ gas chromatographic approaches although liquid chromatographic methods are beginning to gain favor and are likely to become more widely utilized with improvements in detector technology. A variety of hyphenated techniques including LC–MS and 2DGC have also seen wider application. This trend is also likely to grow in the future.

References

1. ASTM D 6751. Standard specification for biodiesel fuel blend stock (B100) for middle distillate fuels. ASTM International. West Conshohocken PA.

2. EN 14214. Automotive fuels—Fatty acid methyl esters (FAME) for diesel engines—Requirements and test methods.
3. M.R. Monteiro, A.R.P Ambrozin, L.M. Liao, and A. G. Ferreira. Critical review on analytical methods for biodiesel characterization. *Talanta* **77:** 593–605 (2008).
4. G. Knothe. Analytical methods used in the production and fuel quality assessment of biodiesel. *Transactions of the ASAE* **44:** 193–200 (2001).
5. G. Knothe. Analyzing biodiesel: standards and other methods. *J. Am. Oil Chem. Soc.* **83:** 823–833 (2006).
6. G. Knothe. *The Biodiesel Handbook*, G. Knothe, J. Van Gerpen and J. Krahl, Eds. AOCS Press, Champaign, IL, 2005, pp. 149–164.
7. D. Bajpai and V. K. Tyagi. Biodiesel: source, production, composition, properties and its benefits. *J. Oleo Sci.* **55:** 487–502 (2006).
8. A. Cert, W. Moreda, and M. C. Perez-Camino. Chromatographic analysis of minor constituents in vegetable oils. *J. Chromatogr. A* **881:** 131–148 (2000).
9. C. Li, H. Yang, L. Wang, and S. Tian. Application of gas chromatography of biodiesel processing. *Sepu* **24:** 524–528 (2006).
10. L. Wang, S. Tian, C. Li, and Q. Wang. Application of analysis technology in biodiesel production and research. *Lianyou Jishu Yu Gongcheng.* **35:** 42–46 (2005).
11. A.S. Ramadhas. "Biodiesel production technologies and substrates". *Handbook of Plant-Based Biofuels*. A. Pandey, Ed. CRC Press, Cleveland, 2008, pp. 183–198.
12. EN 14110. Fat and oil derivatives—Fatty acid methyl esters (FAME)—Determination of methanol content.
13. P. Bondioli, C. Mariani, E. Fedeli, A. M. Gomez, and S. Veronese. Vegetable oil derivatives as diesel fuel substitutes. Analytical aspects. Note 3: determination of methanol. *Rivista Italiana delle Sostanze Grasse* **69:** 467–469 (1992).
14. M. Mittelbach, G. Roth, and A. Bergmann. Simultaneous gas chromatographic determination of methanol and free glycerol in biodiesel. *Chromatographia* **42:** 431–434 (1996).
15. M.C. Paraschivescu, E.G. Alley, W.T. French, R. Hernandez, and K. Armburst. Determination of methanol in biodiesel by headspace solid phase extraction. *Bioresour. Technol.* **99:** 5901–5905 (2008).
16. G. Arzamendi, E. Arguinarena, I. Campo, and L.M. Gandia. Monitoring of biodiesel production: simultaneous analysis of the transesterification products using size-exclusion chromatography. *Chem. Eng. J.* **122:** 31–40 (2006).
17. J. Campo, A. Gonzalez, and R. Rodriguez. Alternative method for analysis of methanol in biodiesel. Gulf Coast Conference. Galveston, TX, January 20, 2008.
18. R. Bergeron, BP, personal communication. June, 2009.
19. R.E. Pauls and R.W. McCoy. Gas and liquid chromatographic analyses of methanol, ethanol, t-butanol and methyl t-butyl ether in gasoline. *J. Chromatogr. Sci.* **19:** 558–561 (1981).
20. D 6584. Standard test method for determination of free and total glycerin in B-100 biodiesel methyl esters by gas chromatography. ASTM International. West Conshohocken PA.
21. EN 14105. Fat and oil derivatives-Fatty acid methyl esters (FAME)-Determination of free and total glycerol and mono-, di- and triglyceride contents.
22. M. Taylor, R. Whitney and M. Waller. GC-2010 analysis of biodiesel for free and total glycerins by ASTM D 6584 with near on-column injection. *LC/GC North America.* **25(2):** 61 (2007).
23. T. Ruppel and G. Hall. Free and total glycerin in biodiesel by gas chromatography. *LC/GC Europe.* **March 2:** 45 (2007).
24. J. Chang, R. Biniakewitz, and G. Harkey. Determination of free and total glycerin in B-100 biodiesel via method ASTM D 6584. *LC/GC North America.* **25(9):** 62 (2007).
25. K. Kelly, S. Countryman, and N. Nguyen. Reliable analysis of glycerin in biodiesel using a high temperature non-metal GC column. *LC/GC North America.* **26(2):** 60 (2007).
26. M. Fausto, D. Cavagnino, and A. Cadoppi. Determination of free and total glycerin in pure biodiesel (B100) by GC in compliance with EN14105. *LC/GC Europe.* **Dec. 2:** 24 (2007).
27. P. Bondioli, C. Mariani, A. Lanzani, E. Fedeli, and S. Veronese. Vegetable oil derivatives as diesel fuel substitutes. Analytical aspects. Note 2: determination of free glycerol. *Rivista Italiana delle Sostanze Grasse* **69:** 7–9 (1992).
28. M. Mittelbach. Diesel fuel derived from vegetable oils: Gas chromatographic determination of free glycerol in transesterified vegetable oils. *Chromatographia* **37:** 623–626 (1993).
29. M. Sala and P. Bondioli. Analytical determination of glycerol. Experimental considerations. *Rivista Italiana delle Sostanze Grasse* **75:** 305–308 (1998).
30. P. Bondioli and L. Della Bella. An alternative spectrophotometric method for the determination of free glycerol in biodiesel. *Eur. J. Lipid Sci. Tech.* **107:** 153–157 (2005).
31. K. Bansal, J. McCrady, A. Hansen, and K. Bhalerao. Thin layer chromatography and image analysis to detect glycerol in biodiesel. *Fuel* **87:** 3369–3372 (2008).
32. M. Hajek, F. Skopal, and J. Machek. Determination of free glycerol in biodiesel. *Eur. J. Lipid Sci. Tech.* **108:** 666–669 (2006).
33. T.A. Foglia, K.C. Jones, R. Moreau, M.J. Haas, and W.N. Marmer. Biodiesel HPLC Methods: Determination of glycerol and blend level. Biodiesel Technical Workshop. Tucson, AZ, November 7–9, 2006.
34. B. Freedman, 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).
35. C. Mariani, P. Bondioli, S. Venturini, and E. Fedeli. Vegetable oil derivatives as diesel fuel substitutes. Analytical aspects. 1: Determination of methyl esters, mono-, di- and triglycerides. *Rivista Italiana delle Sostanze Grasse* **68:** 549–551 (1991).
36. C. Plank 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.* **15:** 609–612 (1992).
37. C. Plank 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).
38. R. Wawrzyniak and W. Wasiak. Determination of acylglycerols in diesel oils by GC. Toxicol. *Mech. Method* **18:** 531–536 (2008).
39. M. Lechner, 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).
40. A.B. Fialkov, A. Gordin, and A. Amirav. Hydrocarbons and fuels analyses with the supersonic gas chromatography mass spectrometry-the novel concept of isomer abundance analysis. *J. Chromatogr. A* **1195:** 127–135 (2008).
41. T.A. Foglia, K.C. Jones, A. Nunez, J.G. Phillips, and M. Mittelbach. Comparison of chromatographic methods for the determination of bound glycerol in biodiesel. *Chromatographia* **60:** 305–311 (2004).
42. T.A. Foglia and K.C. Jones. Quantitation of neutral lipid mixtures using high performance liquid chromatography with light scattering detection. *J. Liquid Chromatogr.* **20:** 1829–1838 (1997).
43. M Holcapek, P. Jandera, J. Fischer, and B. Prokes. Analytical monitoring of the production of biodiesel by high-performance liquid chromatography with various detection methods. *J. Chromatogr. A* **858:** 13–31 (1999).
44. M. Holcapek, P. Jandera, and J. Fischer. Analysis of acylglycerols and methyl esters of fatty acids in vegetable oils and in biodiesel. *Critical Reviews in Analytical Chemistry* **31:** 53–56 (2001).
45. M. Holcapek and P. Jandera. Analysis of acylglycerols and fatty acid methyl esters using HPLC/APCI-MS. *Advances in Mass Spectrometry.* E. Gelpi, Ed. John Wiley and Sons, New York, NY, 2001, pp. 583-585.
46. M. Holcapek, P. Jandera, P. Zderadicka, and L. Hruba. Characterization of triacylglycerol and diacylglycerol composition of plant oils using high-performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. *J. Chromatogr. A* **1010:** 195–215 (2003).
47. M. Holcapek, M. Lisa, P. Jandera, and N. Kabatova. Quantitation of triacylglycerols in plant oils using HPLC with APCI-MS, evaporative light scattering, and UV detection. *J. Sep. Sci.* **28:** 1315–1333 (2005).
48. G. Di Nicola, M. Pacetti, F. Polonara, G. Santori, and R. Stryjek. Development and optimization of a method for analyzing biodiesel mixtures with non-aqueous reversed phase liquid chromatography. *J. Chromatogr. A* **1190:** 120–126 (2008).
49. A. Tuerkan and S. Kalay. Monitoring lipase-catalyzed methanolysis of sunflower oil by reversed-phase high-performance liquid chromatography: Elucidation of the mechanisms of lipases. *J. Chromatogr. A* **1127:** 34–44 (2006).
50. G. Santori, A. Arteconi, G. Di Nicola, M. Moglie, and R. Stryjek. Quantitation of compounds in biodiesel mixtures with reversed-phase liquid chromatography. *Energy Fuels* **23:** 3783–3789 (2009).
51. Metrohm Application Note P-38. Free and total glycerin in biodiesel and biodiesel blends. Riverview, FL.
52. J. Lubkowitz, Separation Systems. Personal communication, March 7, 2010.
53 B. Trathnigg and M. Mittelbach. Analysis of triglyceride methanolysis mixtures with isocratic HPLC with density detection. *J. Liquid Chromatogr.* **13:** 95–105 (1990).
54. R. Fillieres, B. Benjelloun-Mlayah, and M. Delmas. Ethanolysis of rapeseed oil: quantitation of ethyl esters, mono-, di-, and triglycerides and glycerol by high performance size-exclusion chromatography. *J. Am. Oil Chem. Soc.* **72:** 427–432 (1995).
55. D. Darnoko, M. Cheryan, and E.G. Perkins. Analysis of vegetable oil transesterification products by gel permeation chromatography. *J. Liquid Chromatogr.* **23:** 2327–2335 (2000).
56. M.A. Dube, S. Zheng, D.D. McLean, and M. Kates. A comparison of attenuated total reflectance FTIR spectroscopy and GPC for monitoring biodiesel production. *J. Am. Oil Chem. Soc.* **81:** 599–603 (2004).
57. K. Kittirattanapiboon and K. Krisnangkura. Separation of acylglycerols, FAME and FFA in biodiesel by size exclusion chromatography. *Eur. J. Lipid Sci. Tech.* **110:** 422–427 (2008).
58. B. Freedman, 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).
59. J.D. Fontana, G. Zagonel, W.W. Vechiatto, B.J. Costa, J.C. Laurindo, R. Fontana, L. Pellisson, B.H. Jorge, and F. M. Lancas. Simple TLC-screening of acylglycerol levels in biodiesel as an alternative to GC determination. *J. Chromatogr. Sci.* **47:** 844–846 (2009).
60. J. Cole, J.L. Lefler, and R. Chen. Fast separation of FFA, FAME and Glycerol for biodiesel analysis by supercritical fluid chromatography. *LC/GC Europe.* **March 2:** 54 (2008).
61. M.L. Lee, F.J. Yang, and K.D. Bartle. *Open Tubular Column Gas Chromatography.* John Wiley. New York, 1984.
62. AOCS Official Method Ce 1h-05. Determination of cis-, trans-, saturated, monounsaturated and polyunsaturated fatty acids in vegetable or non-ruminant animal oils and fats by capillary GLC. American Oil Chemists Society, Urbana, IL, 2005.
63. W.M.N. Ratnayake, S. L. Hansen and M. P. Kennedy. Evaluation of the CP-Sil 88 and SP-2560 GC columns used in the recently approved AOCS official method Ce 1h-05. Determination of cis-, trans-, saturated, monounsaturated and polyunsaturated fatty acids in vegetable or non-ruminant animal oils and fats by capillary GLC method. *J. Am. Oil Chem. Soc.* **83:** 475–488 (2006).

64. P. Delmonte and J.I. Rader. Evaluation of gas chromatographic methods for the determination of trans fat. *Anal. Bioanal. Chem.* **389:** 77–85 (2007).
65. K. Yamamoto, A. Kinoshita, and A. Shibahara. Gas chromatographic separation of fatty acid methyl esters on weakly polar capillary columns. *J. Chromatogr. A* **1182:** 132–135 (2008).
66. W. Kiridena, J. Qian, W.W. Koziol, and C.F. Poole. Evaluation of the separation characteristics of application-specific (fatty acid methyl esters) open-tubular columns for gas chromatography. *J. Sep. Sci.* **30:** 740–745 (2007).
67. J. Harynuk, P.M. Wynne, and P.J. Marriott. Evaluation of new stationary phases for the separation of fatty acid methyl esters. *Chromatographia* **63:** S61–S66 (2006).
68. A. Ruiz-Rodriguez, G. Reglero, and E. Ibanez. Recent trends in the advanced analysis of bioactive fatty acids. *J. Pharm. Biomed. Anal.* **51:** 305–326 (2010).
69. EN 14103. Fat and oil derivatives—Fatty acid methyl esters (FAME)—Determination of ester and linolenic acid methyl ester contents.
70. S. Schober, I. Seidl, and M. Mittelbach. Ester content evaluation in biodiesel from animal fats and lauric oils. *Eur. J. Lipid Sci. Tech.* **108:** 309–314 (2006).
71. H. Wu, F. Ling, S. Wang, and M. Qian. A measurement of fatty acid methyl esters in palm bio-diesel by GC-MS. *Dangdai Huagong* **36:** 538–540 (2007).
72. Y. Fu, Y. Zu, L. Wang, N. Zhang, W. Liu, S. Li, and S. Zhang. Determination of fatty acid methyl esters in biodiesel produced from yellow horn oil by LC. *Chromatographia* **67:** 9–14 (2008).
73. Y. Li, G. Bao, and H. Wang. Determination of eleven fatty acids and fatty acid methyl esters in biodiesel using ultra performance liquid chromatography. *Sepu* **26:** 494–498 (2008).
74. Y. Chen, P. Lu, J. Lu, B. Xiao, and M. Xiao. Analysis of biodiesel from waste cooking oil by GC-MS. *Huaxue Yu Shengwu Gongcheng* **24:** 72–74 (2007).
75. X. Zhang, J. Fang, and L. Bi. Study on constituent analysis of fatty acid methyl esters in biodiesel by GC-MS methods. *Shengwuzhi Huaxue Gongcheng* **42:** 23–25 (2008).
76. J.S. Buyer. *Lipid Analysis and Lipidomics*. M.M. Mossoba, J.K.G. Kramer, J.T. Brenna, and R.E. McDonald, Eds. AOAC Press, Champaign, IL, 2006, pp. 271–283.
77. A. Shibahara, K. Yamamoto, and A. Kinoshita. High-speed analysis of the major components of fatty acid methyl esters by capillary gas chromatography. *Lipid Technol.* **20:** 88–90 (2008).
78. M.A. Masood and N. Salem. High-throughput analysis of plasma fatty acid methyl esters employing robotic transesterification and fast gas chromatography. *Lipids* **43:** 171–180 (2008).
79. L. Mondello, P.Q. Tranchida, P. Dugo, and G. Dugo. Rapid micro-scale preparation and very fast gas chromatographic separation of cod liver oil fatty acid methyl esters. *J. Pharm. Biomed. Anal.* **41:** 1566–1570 (2006).
80. K. D. Stark and N. Salem. Fast gas chromatography for the identification of fatty acid methyl esters from mammalian samples. *Lipid Technol.* **17:** 181-185 (2005).
81. P. Q. Tranchida, M. Mondello, D. Sciarrone, P. Dugo, G. Dugo and L. Mondello. Evaluation of use of a very short polar microbore column segment in high-speed gas chromatography analysis. *J. Sep. Sci.* **31:** 2634-2639 (2008).
82. R. E. Pauls, R. Shea and T. Lynch. Advanced characterization of biodiesel. ACS Great Lakes Meeting, Chicago, IL, May, 2009.
83. J. B. Phillips and J. Xu. Comprehensive multi-dimensional gas chromatography. *J. Chromatogr. A* **703:** 327-334 (1995).
84. *Multidimensional Chromatography*. L. Mondello, A.C. Lewis, and K.D. Bartle, Eds. John Wiley and Sons, New York, 2002.
85. P. Q. Tranchida, P. Donato, G. Dugo, L. Mondello, and P. Dugo. Comprehensive chromatographic methods for the analysis of lipids. *Trends Anal. Chem.* **26:** 191-205 (2007).
86. W. Tiyapongpattana, P. Wilairat, and P.J. Marriott. Characterization of biodiesel and biodiesel blends using comprehensive two-dimensional gas chromatography. *J. Sep. Sci.* **31:** 2640–2649 (2008).
87. P.Q. Tranchida, P. Dugo, G. Dugo, and L. Mondello. *Comprehensive Two Dimensional Gas Chromatography.* L. Ramos, Ed. Elsevier, Amsterdam, 2009, pp. 215–242.
88. H. de Geus, I. Aidos, J. de Boer, J. B. Luten, and U. A. Th. Brinkman. Characterization of fatty acids in biological oil samples using comprehensive multidimensional gas chromatography. *J. Chromatogr. A* **910:** 95–103 (2001).
89. T. Hyotylainen, M. Kallio, M. Lehtonen, S. Lintonen, P. Perajoki, M. Jussila, and M. Riekkola. Comprehensive two-dimensional gas chromatography in the analysis of dietary fatty acids. *J. Sep. Sci.* **27:** 459–467 (2004).
90. R.J. Western, S.S.G. Lau, P.J. Marriott, and P.D. Nichols. Positional and geometric isomer separation of FAME by comprehensive 2-D GC. *Lipids* **37:** 715–724 (2002).
91. L. Mondello, A. Casilli, P.Q. Tranchida, P. Dugo, and G. Dugo. Detailed analysis and group-type separation of natural fats and oils using comprehensive two-dimensional gas chromatography. *J. Chromatogr. A* **1019:** 187–196 (2003).
92. P.Q. Tranchida, A. Giannino, M. Mondello, D. Sciarrone, P. Dugo, G. Dugo, and L. Mondello. Elucidation of fatty acid profiles in vegetable oils exploiting group-type patterning and enhanced sensitivity of comprehensive two-dimensional gas chromatography. *J. Sep. Sci.* **31:** 1797–1802 (2008).
93. S. de Koning, H. Janssen, M. van Deursen, and U.A. Th. Brinkman. Automated on-line comprehensive two-dimensional LCxGC and LCxGC-ToF MS: Instrument design and application to edible oil and fat analysis. *J. Sep. Sci.* **27:** 397–409 (2004).
94. E.J.C. van der Klift, G. Vivo-Truyols, F.W. Claassen, F.L. van Holthoon, and T.A. van Beek. Comprehensive two-dimensional liquid chromatography with ultraviolet, evaporative light scattering and mass spectrometric detection of triacylglycerols in corn oil. *J. Chromatogr. A* **1178:** 43–55 (2008).
95. P. Dugo, F. Cacciola, T. Kumm, G. Dugo, and L. Mondello. Comprehensive multidimensional liquid chromatography: Theory and applications. *J. Chromatogr. A* **1184:** 353–368 (2008).
96. L. Mondello, P.Q. Tranchida, V. Stanek, P. Jandera, G. Dugo, and P. Dugo. Silver-ion reversed phase comprehensive two-dimensional liquid chromatography combined with mass spectrometric detection in lipidic food analysis. *J. Chromatogr. A* **1086:** 91–98 (2005).
97. I. Francois and P. Sandra. Comprehensive supercritical fluid chromatography x reversed phase liquid chromatography for the analysis of the fatty acids in fish oil. *J. Chromatogr. A* **1216:** 4005–4012 (2009).
98. Y. Hirata and I. Sogabe. Separation of fatty acid methyl esters by comprehensive two-dimensional supercritical fluid chromatography. *Anal. Bioanal. Chem.* **378:** 1999–2003 (2004).
99. L. Hejazi, D. Ebrahimi, M. Guilhaus, and D. B. Hibbert. Determination of the composition of fatty acid mixtures using GC x FI-MS: A comprehensive two-dimensional separation approach. *Anal. Chem.* **81:** 1450–1458 (2009).
100. F.C. Wang, K. Qian, and L. A. Green. GC x MS of diesel: A two-dimensional separation approach. *Anal. Chem.* **77:** 2777–2785 (2005).
101. L. Mondello, P.Q. Tranchida, P. Dugo, and G. Dugo. Comprehensive two-dimensional gas chromatography-mass spectrometry: A review. *Mass Spectrom. Reviews.* **27:** 101–124 (2008).
102. C. Hartig. Rapid identification of fatty acid methyl esters using a multidimensional gas chromatography-mass spectrometry database. *J. Chromatogr. A* **1177:** 159–169 (2008).
103. *HPLC of Acyl Lipids*. J. Lin and T.A. McKeon, Eds. HNB Publishing, New York, 2005.
104. R.O. Adlof. *Lipid Analysis and Lipidomics*. M.M. Mossoba, J.K.G. Kramer, J.T. Brenna and R.E. McDonald, Eds. AOAC Press, Champaign, IL, 2006, pp. 175–190.
105. P. Delmonte, J.K.G. Kramer, and M.P. Yurawecz. *Lipid Analysis and Lipidomics*. M.M. Mossoba, J.K.G. Kramer, J.T. Brenna, and R.E. McDonald, Eds. AOAC Press, Champaign, IL, 2006, pp. 191–204.
106. K. Gaudin, T. Hanai, P. Chaminade, and A. Baillet. Retention behavior of polyunsaturated fatty acid methyl esters on porous graphitic carbon. *J. Chromatogr. A* **1157:** 56–64 (2007).
107. R.M. Smith, M. Hyytiainen, A.V. Felipe, and P.M. Morris. High resolution packed column supercritical fluid chromatography of fatty acid methyl esters. *J. Sep. Sci.* **24:** 208–212 (2001).
108. D.G. Hayes. *Lipid Analysis and Lipidomics*. M.M. Mossoba, J.K.G. Kramer, J.T. Brenna, and R.E. McDonald, Eds. AOAC Press, Champaign, IL, 2006, pp. 239–260.
109. EN 14078. Liquid petroleum products—Determination of fatty acid methyl esters (FAME) in middle distillates—Infrared spectroscopy method.
110. J.S. Oliveira, R. Montalvao, L. Daher, P.A. Suarez, and J.C. Rubim. Determination of methyl ester contents in biodiesel blends by FTIR-ATR and FTNIR spectroscopies. *Talanta* **69:** 1278–1284 (2006).
111. G. Knothe. Determining the blend level of mixtures of biodiesel with convention diesel fuel by fiber-optic near infrared spectroscopy and H nuclear magnetic resonance spectroscopy. *J. Am. Oil Chem. Soc.* **78:** 1025–1028 (2001).
112. M.F. Pimentel, 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).
113. F.C.C. Oliveira, C.R.R. Brandao, H.F. Ramalho. L.A. da Costa, P.A Suarez, and J.C. Rubim. Adulteration of diesel/biodiesel blends by vegetable oil as determined by Fourier transform (FT) near infrared spectrometry and FT-Raman spectroscopy. *Anal. Chim. Acta* **587:** 194–199 (2007).
114. I. K. de Oliveria, W.F. de Carvalho Rocha, and R.J. Poppi. Application of near infrared spectroscopy and multivariate control charts for monitoring biodiesel blends. *Anal. Chim. Acta* **642:** 217–221 (2009).
115. P. Bondioli, 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 a mixture. *Rivista Italiana dell Sostanze Grasse* **71:** 287–289 (1994).
116. EN 14331. Liquid petroleum products-Separation and characterization of fatty acid methyl esters (FAME) from middle distillates—Liquid chromatography (LC)/gas chromatography (GC) method.
117. R. Wawrzyniak, W. Wasiak, and M. Frackowiak. Determination of methyl esters in diesel oils by gas chromatography—validation of the method. *Chem. Pap.* **59:** 449–452 (2005).
118. F. Adam, F. Bertoncini, V. Coupard, N. Charon, D. Thiebaut, D. Espinat, and M. Hennion. Using comprehensive two-dimensional gas chromatography for the analysis of oxygenates in middle distillates I. Determination of the nature of biodiesels blend in diesel fuel. *J. Chromatogr. A* **1186:** 236–244 (2008).
119. S.K. Seeley, J.D. McCurry, and J.V. Seeley. Characterizing biodiesel/petroleum mixtures with comprehensive two-dimensional gas chromatography. 40th Central Regional Meeting of the ACS. Columbus OH, June 10–14. (2008).
120. J.V. Seeley, S.K. Seeley, E.K. Libby, and J. D. McCurry. Analysis of biodiesel/petroleum diesel blends with comprehensive two-dimensional gas chromatography. *J. Chromatogr. Sci.* **45:** 650–656 (2007).
121. J.D. McCurry and C. Wang. Improving the analysis of biodiesel using capillary flow technology. *LC/GC North America* **June:** 48–49 (2007).
122. H. Tang, N. Abunasser, A. Wang, B.R. Clark, K. Wadumesthrige, S. Zeng, M. Kim. S.O. Salley, G. Hirschlieb, J. Wilson, and K.Y. S. Ng. Quality survey of biodiesel blends sold at retail stations. *Fuel* **87:** 2951–2955 (2008).

123. T.A. Foglia, K.C. Jones, and J.G. Phillips. Determination of biodiesel and triacylglycerols in diesel fuel by LC. *Chromatographia* **62:** 115–119 (2005).
124. M. Kaminski, 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).
125. J.W. Diehl and F.P. DiSanzo. Determination of total biodiesel fatty acid methyl, ethyl esters and hydrocarbon types in diesel fuels by supercritical fluid chromatography-flame ionization detection. *J. Chromatogr. Sci.* **45:** 690–693 (2007).
126. C.M. White. The use of supercritical fluid chromatography in biodiesel analysis. Gulf Coast Conference. Galveston, TX, Oct. 13, 2009.
127. T. Lynch and A. Ttofi. Measurement of low levels of FAME in Aviation Fuel by GC-MS. Biofuels: Measurement & Analysis Symposium 2008. Windsor, UK, April 15–16, 2008.
128. J.V. Seeley, C.T. Bates, J.D. McCurry, and S.K. Seeley. Comprehensive two-dimensional gas chromatography analysis of aviation fuel contaminated with biodiesel. Gulf Coast Conference. Galveston, TX, Oct. 13, 2009.
129. J. McCurry, B. Quimby, and R. Ong. Improving the analysis of FAMEs in jet fuel using GC/MS combined with heart cutting 2-D GC. Gulf Coast Conference. Galveston, TX, Oct 13, 2009.
130. S.J. Hartman, O. Seiler, L. Owen, and P. Spitteler. A rapid laboratory analytical method for detecting low levels of FAME in jet fuel: method development and preliminary industry study results (UK). 11th International Conference on Stability, Handling and Use of Liquid Fuels. Prague, Czech Republic. October 18–22, 2009.
131. P. Spitteler, R. Shaw, M. Sherratt, and S. Hartman. The development and use of flow analysis by FTIR spectroscopy in a rapid screening test method for measuring traces of fatty acid methyl esters in aviation turbine fuels. 11th International Conference on Stability, Handling and Use of Liquid Fuels. Prague, Czech Republic. October 18-22, 2009.
132. H. Read and R. Hooks. Analytical test method development-measurement of FAME in aviation turbine fuel. The preventing jet fuel contamination by FAME – Best practice in the distribution system. Energy Institute. London, UK, January 20, 2009.
133. A.A. Durant, M. Dumont, and S.S. Narine. In situ silylation for the multicomponent analysis of canola oil by-products by gas chromatography-mass spectrometry. *Anal. Chim. Acta* **559:** 227–233 (2006).
134. F.D. Gunstone, J.L. Harwood, and F. B. Padley. *The Lipid Handbook* 2nd Edition, Chapman & Hall, London, 1994.
135. J.A. Bowden, D.M. Colosi, W.L. Stutts, D.C. Mora-Montero, T.J. Garrett, and R.A. Yost. Enhanced analysis of steroids by gas chromatography/mass spectrometry using microwave-accelerated derivatization. *Anal. Chem.* **81:** 6725–6734 (2009).
136. C. Plank 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).
137. C. Plank 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).
138. C. Plank and E. Lorbeer. Minor components in vegetable oil methyl esters I. Sterols in rape seed oil methyl ester. *Fett Wissenschaft Technol.* **96:** 379–386 (1994).
139. H. Schwartz, V. Ollilainen, V. Piironen, and A. Lampi. Tocopherol, tocotrienol and plant sterol contents of vegetable oils and industrial fats. *J. Food Comp. Anal.* **21:** 152–161 (2008).
140. J. Gruszka and J. Kruk. RP-LC for determination of plastochromanol, tocotrienols and tocopherols in plant oils. *Chromatographia* **66:** 909–913 (2007).
141. S. Bostyn, F. Duval-Onen, C. Porte, J. Coic, and H. Fauduet. Kinetic modeling of the degradation of the alpha tocopherol in biodiesel-rape methyl ester. *Bioresour. Technol.* **99:** 6439–6445 (2008).
142. A. Frohlich and S. Schober. The influence of tocopherols on the oxidation stability of methyl esters. *J. Am. Oil Chem. Soc.* **84:** 579–585 (2007).
143. L. Pfalzgraf, I. Lee, T. Haines, E. Powers, J. Hammer, S. Fenwick, and G. Poppe. Identification of sterol glucosides in biodiesel and their effect on filterability. 98th AOCS Annual Meeting. Quebec City, Canada, May 13–16, 2007.
144. I. Lee, L.M. Pfalzgraf, G.B. Poppe, E. Powers, and T. Haines. The role of sterol glucosides on filter plugging. *Biodiesel Magazine*. April, 2007.
145. M. Foster, R. George, and M. Winward. Biodiesel quality-a petroleum company's perspective. National Biodiesel Technical Workshop. Tucson, AZ, Nov. 7–9 (2006).
146. R.C. Shea, R.E. Pauls, P.S. Munowitz, and M. Foster. Characterization of B100 biodiesel and biodiesel precipitants by GCMS and LCMS. Am. Soc. Mass Spectrom. Denver, CO, June 2008.
147. V. Van Hoed, N. Zyaykina, W. De Greyt, J. Maes, R. Verhe, and K. Demeestere. Identification and occurrence of steryl glucosides in palm and soy biodiesel. *J. Am. Oil Chem. Soc.* **85:** 701–709 (2008).
148. P. Bondioli, N. Cortesi, and C. Mariani. Identification and quantification of steryl glucosides in biodiesel. *Eur. J. Lipid Sci. Tech.* **110:** 120–126 (2008).
149. R.A. Moreau, K.M. Scott, and M.J. Haas. Identification and quantification of steryl glucosides in precipitates from commercial biodiesel. *J. Am. Oil Chem. Soc.* **85:** 761–770 (2008).
150. H. Tang, S.O. Salley, and K.Y.S. Ng. Fuel properties and precipitate formation at low temperature in soy-, cottonseed-, and poultry fat-based biodiesel blends. *Fuel* **87:** 3006–3017 (2008).
151. H. Tang. R.C. De Guzman, S.O. Salley, and K.Y.S. Ng. Formation of insolubles in palm oil-, yellow grease- and soybean oil-based biodiesel blends after cold soaking at 4°C. *J. Am. Oil Chem. Soc.* **85:** 1173–1182 (2008).
152. K.J. Byrne, G.H. Reazin, and A.A. Andreasen. High pressure liquid chromatographic determination of β-sitosterol and β-sitosterol-D-glucoside in whiskey. *J. Assoc. Off. Anal. Chem.* **64:** 181–185 (1981).
153. B.A. Knights. Gas-liquid chromatography of sterol glycosides. *Anal. Letters* **6:** 495–503 (1973).
154. K.M. Phillips, D.M. Ruggio, and M. Ashraf-Khorassani. Analysis of steryl glucosides in foods and dietary supplements by solid-phase extraction and gas chromatography. *J. Food Lipids* **12:** 124–140 (2005).
155. F. Lacoste, F. Dejean, H. Griffon, and C. Rouquette. Quantification of free and esterified steryl glucosides in vegetable oils and biodiesel. *Eur. J. Lipid Sci. Technol.* **111:** 822–828 (2009).
156. R. Shea and R.E. Pauls. Characterization of sterol glucosides found in B100 biodiesels by gas and liquid chromatography/mass spectrometry. Am. Soc. Mass Spectrom. Philadelphia, PA, June 2009.
157. I. Eide and K. Zahlsen. Chemical fingerprinting of biodiesel using electrospray mass spectrometry and chemometrics: Characterization, discrimination, identification and quantification in petrodiesel. *Energy and Fuels* **21:** 3702–3708 (2007).
158. R.R. Catharino, H.M.S. Milagre, S.A. Saraiva, C.M. Garcia, U. Schuchardt, M.N. Eberlin, R. Augusti, R.C.L. Pereira, M.J.R. Guimaraes, G.F. de Sa, J.M.R. Caixeiro, and V. De Souza. Biodiesel typification and quality control by direct infusion electrospray ionization mass spectrometry fingerprinting. *Energy and Fuels* **21:** 3698–3701 (2007).
159. P.V. Abdelnur, L.S. Eberlin, G.F. de Sa, V. de Souza, and M.N. Eberlin. Single-shot biodiesel analysis: Nearly instantaneous typification and quality control solely by ambient mass spectrometry. *Anal. Chem.* **80:** 7882–7886. (2008).
160. C. Wu, D.R. Ifa, N.E. Manicke, and R. G. Cooks. Rapid direct analysis of cholesterol by charge labeling in reactive desorption electrospray ionization. *Anal. Chem.* **81:** 7618–7624 (2009).
161. R. Aparicio and R. Aparicio-Ruiz. Authentication of vegetable oils by chromatographic techniques. *J. Chromatogr. A* **881:** 93–104 (2000).
162. L. Fan, N. Wu, Q. Huo, and M. Wang. Discrimination of five kinds of vegetable oil by analyzing the characteristics of fatty acids. *Henan Gongye Daxue Xuebao.* **28:** 26–27 (2007).
163. M.V. Ruiz-Mendez, S. Marmesat, A. Liotta, and M.C. Dobarganes. Analysis of used frying fats for the production of biodiesel. *Grasas Y Aceites* **59:** 45–50 (2008).
164. D.S. Giordani, A.F. Siqueira, M.L.C. P. Silva, P.C. Oliveira. and H.F. de Castro. Identification of the biodiesel source using an electronic nose. *Energy and Fuels* **22:** 2743–2747 (2008).
165. D 975. Standard Specification for Diesel Fuel Oils. ASTM International. West Conshohocken PA.
166. D 2887. Standard Test Method for Boiling Range Distribution of Petroleum Fractions by Gas Chromatography. ASTM International. West Conshohocken PA.
167. ASTM D 5186. Standard Test Method for Determination of Aromatic Content and Polynuclear Aromatic Content of Diesel Fuels and Aviation Turbine Fuels by Supercritical Fluid Chromatography. ASTM International. West Conshohocken PA.
168. ASTM D 6591 Standard Test Method for Determination of Aromatic Hydrocarbon Types in Middle Distillates-High Performance Liquid Chromatography Method with Refractive Index Detection. ASTM International. West Conshohocken PA.
169. H. Read, Rothsay Consulting, personal communication, October, 2009.
170. C. Bachler, S. Schober, and M. Mittlebach. Simulated distillation for biofuel analysis. *Energy and Fuels* **24:** 2086–2090 (2010).

Manuscript received April 24, 2010;
revision received July 20, 2010.