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# Resource Guide to Biofuel Development

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# Biofuel Solutions Resource Guide

## Biodiesel

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Residual Methanol
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# Biodiesel Applications

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GAS CHROMATOGRAPHY

PRODUCT NOTE

# Biodiesel Glycerin and Methanol Analyzer

Biodiesel, a renewable fuel produced from natural oil, is used as either a direct substitute for, or an additive to petroleum-based diesel fuel. Interest in biodiesel has increased as a result of rising oil prices and concerns over future supply.

In the production of biodiesel, free fatty acids (lipids) are catalytically converted to fatty acid methyl esters (FAME) with alcohol, typically methanol. Following this reaction, glycerin, water and residual catalyst must be removed to create a fuel suitable for use in compression ignition (diesel) engines. A number of quality problems can arise if the reaction is incomplete or if by-products are not removed effectively.

To ensure fuel quality, both the European Committee for Standardization (EN 14214) and ASTM International (ASTM D6751) have issued standard test criteria for biodiesel. These standards utilize multiple gas chromatographic (GC) analyses.

The PerkinElmer® EcoAnalytix™ Biodiesel Glycerin and Methanol Analyzer provides a unique solution to test biodiesel using the GC test methods included in both the EN and ASTM standards. The analyzer incorporates a TurboMatrix™ Headspace Sampler coupled to a Clarus® GC.

## Key Benefits

- Clarus GC with dual oven allows analysis of both free and total glycerin as well as residual methanol on a single instrument
- TurboMatrix Headspace Sampler improves productivity and conforms with EN methodology
- Consumables package with calibration standards provides rapid ramp-up of sampling and analysis
- Standard operating procedures allow rapid setup of test methods
- Specific installation and qualification procedures to get the application up and running

The Biodiesel Glycerin and Methanol Analyzer consists of three main components: the TurboMatrix Headspace Sampler, the Clarus GC, and an integrated auxiliary isothermal oven within the GC. The Clarus GC is configured with an autosampler, programmable-on-column injector and two flame ionization detectors. The auxiliary isothermal oven installed within the Clarus GC provides a second temperature-controlled zone for a second chromatographic column. The TurboMatrix Headspace Sampler allows unattended analysis of multiple samples. Figure 1 shows the schematic layout of the 3 components of the system.

The Biodiesel Glycerin and Methanol Analyzer provides the capability to analyze both free and total glycerin (EN 14105, ASTM D6584) as well as residual methanol (EN 14110) with a single, integrated Headspace-GC sampling system.

Figures 2 and 3 (Page 3) demonstrate the chromatography expected when following EN 14105/ASTM D6584 and EN 14110 methodology.

## Trouble-free determination of glycerin and residual methanol in biodiesel

The Clarus GC includes programmable pneumatic control (PPC) which allows computerized control of carrier and detector gases, eliminating time-consuming manual interaction. The integral touch-screen user interface provides real-time monitoring and control of the GC.

The independently-controlled second oven allows the analysis of both glycerin and methanol on the Clarus GC.

*Table 1.* Summary of EN and ASTM methods for biodiesel-quality analysis.

| Method | Analytes | Injection | Analysis Time |
|---|---|---|---|
| EN 14105 | Free and Total Glycerol, Mono-, Di-, and Triglyceride Content | On-Column | 35 min |
| EN 14110 | Residual Methanol | Headspace | < 5 min |
| ASTM D6584 | Free and Total Glycerin | On-Column | 25 min |

The ability to have two independently controlled ovens reduces the operating cost and optimizes laboratory bench space by combining the methods which formerly required two GCs into a single instrument.

Utilization of programmable on-column injection provides high precision for sample injections. The integral liquid autosampler of the Clarus GC is uniquely suited for on-column injections with superior precision.

Use of metal capillary columns in the Analyzer eliminates many of the handling challenges associated with fused-silica capillary columns and improves the reliability of the system.

The EN 14110 method recommends a 45-minute equilibration time for each sample. The TurboMatrix HS-40 and HS-110 Headspace Samplers thermostat up to 12 samples at a time, ensuring the next sample is ready on completion of the previous run, thereby enhancing throughput to meet the EN method's timing specification.

The GC and Headspace instrumentation require calibration with analytical reference-standard compounds. The Biodiesel Glycerin and Methanol Analyzer includes reference-standard compounds for EN 14105, ASTM D6584 and EN 14110 methods. These reference compounds will allow quick and easy system calibration so that the user can be up and running rapidly and focus on validating the quality of the biodiesel. The reference-standard compounds are also available as individual replacement parts which can be reordered to continue operation of the system.

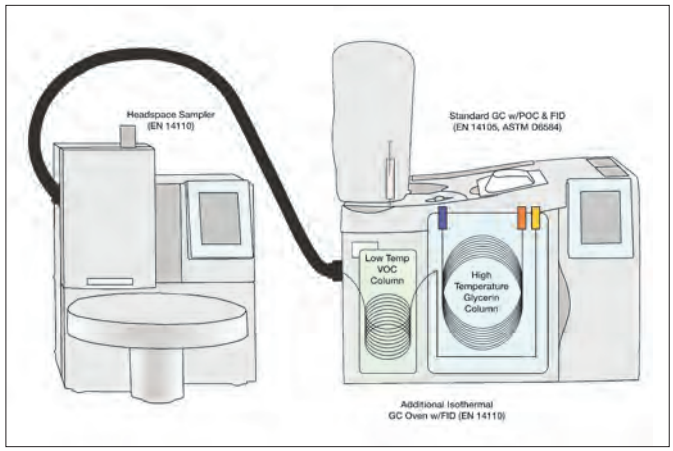


*Figure 1.* Schematic diagram of the EcoAnalytix Biodiesel Glycerin and Methanol Analyzer, highlighting its unique features.

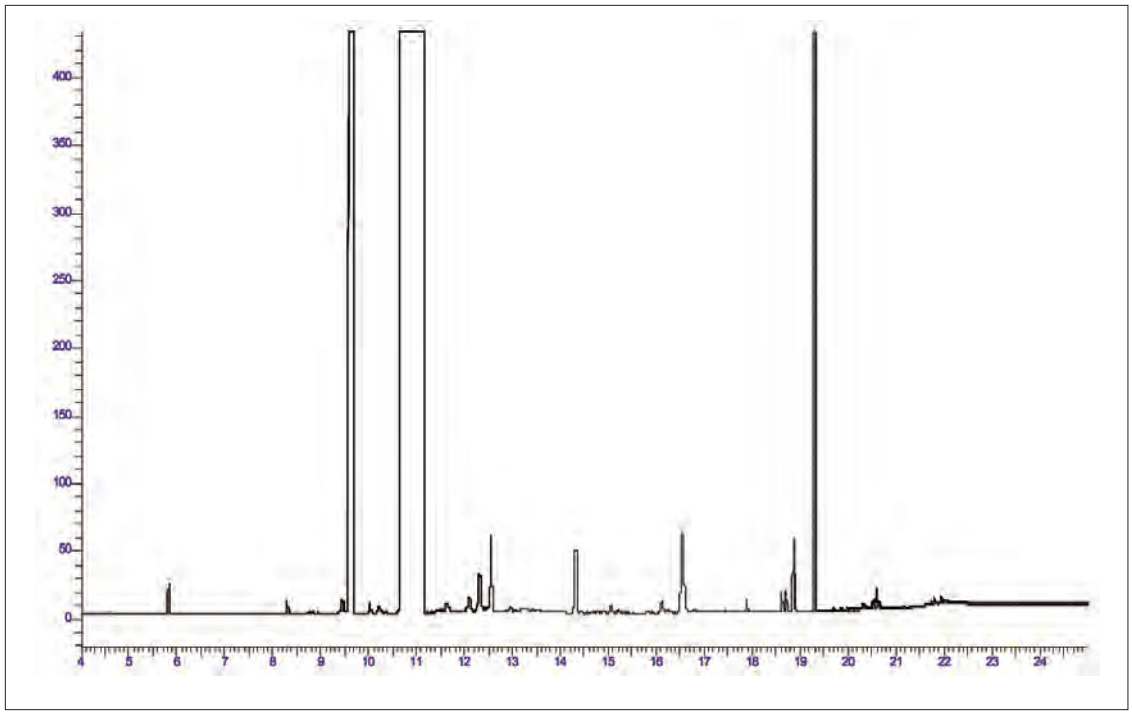


*Figure 2.* Chromatography demonstrating the analysis of a biodiesel sample for glycerin and mono-, di-, triglyceride content.

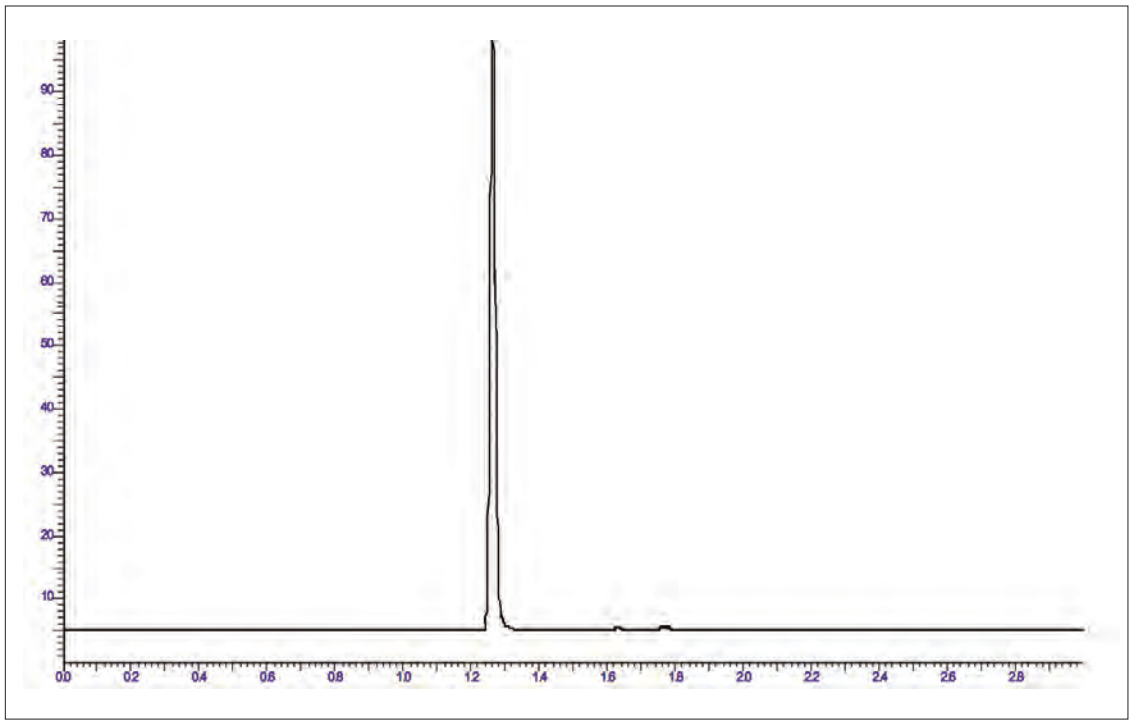


*Figure 3.* Chromatography demonstrating 0.1% methanol in a biodiesel matrix.

Also included with the Biodiesel Glycerin and Methanol Analyzer is an application CD which includes:

- Application notes with background information on biodiesel GC techniques
- Reference data files for chromatograms
- Software methods to control the GC and Headspace instruments
- Processing methods to translate chromatograms into biodiesel-quality information
- Standard operating procedures (SOPs) for sample preparation, calibration, analysis and reporting

PerkinElmer's global service organization completes the process by providing installation and support. This includes qualification that the Biodiesel Glycerin and Methanol Analyzer meets the needs of the application.

## The perfect solution

Complete integration of the Clarus GC with a dual oven, TurboMatrix Headspace Sampler, calibration standards, operating procedures and an application CD makes the PerkinElmer EcoAnalytix Biodiesel Glycerin and Methanol Analyzer the perfect solution to meet the needs of EN 14105, ASTM D6584 and EN 14110 methods in a cost-effective and time-efficient manner.

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# Residual Methanol in B100 Biodiesel by Headspace-Gas Chromatography According to EN 14110

## Introduction

Biodiesel quality specifications in B100 biodiesel are covered in EN 14214 and ASTM D6751-07a. The EN 14110 method specifies headspace-gas chromatography for the determination of residual methanol. Automated headspace sample introduction is recommended in EN 14110 but manual headspace sample introduction is allowed if an internal standard is used. The ASTM method specifies that residual methanol can be determined by either flashpoint at 130 ˚C minimum for ASTM D93, or by less than 0.2% methanol by mass for EN 14110. EN 14110 was adopted by ASTM in 2007, resulting from the lack of an ASTM method for the analysis of methanol in biodiesel.

This application note will focus on automated headspace sample introduction without the use of an internal standard. It will follow the EN 14110 method and then show a modification to simplify and speed up the analysis. Compared to the analysis of free and total glycerin in B100 biodiesel, the analysis of residual methanol is very easy.

## Experimental

Following EN 14110, 5 mL of B100 biodiesel is added to a 22-mL headspace vial, heated at 80 °C for 45 min and 500 µL of headspace vapor is injected to a split injector of the gas chromatograph. A modification of this method uses only 250 µL of sample, heated to 80 °C for 10 minutes.

Instrumentation: PerkinElmer® TurboMatrix™ HS-40 Headspace (HS) sampler, coupled to a PerkinElmer Clarus® 500 Gas Chromatograph (GC) with capillary injector and FID.

GC Column: Several types of GC columns are listed in the EN 14110 method as possibilities. Any column that delivers resolution and a symmetrical peak for methanol is acceptable. The columns included in this application note are:

- 30 m x 0.32 mm x 1.8 µm BAC-1
  (PerkinElmer Part No. N9316579)
- 30 m x 0.28 mm x 3.0 µm Elite-1
  (PerkinElmer Part Nos. N9316025 or N9307067)

This application note demonstrates the analysis following both EN 14110 and the modified analysis for greater sample throughput.

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Calibration standards should be made in a matrix similar to the analytical samples. To accomplish this, a blank biodiesel matrix free of methanol must be created. Wash 100 mL of B100 biodiesel with 50-mL aliquots of water 3 times with agitation to remove methanol. Heat that 100 mL of B100 biodiesel in a 500-mL beaker on a hot plate to 90 ˚C for 2 hours while stirring. This will drive off any traces of methanol, leaving a blank biodiesel suitable for creating calibration standards. Test the blank matrix prior to standard preparation to ensure that no methanol is present. Analyzing a blank headspace vial will verify the lab air is also methanol free. Checking the level of methanol in the lab air is important because trace methanol is often in the atmospheric air of biodiesel production facilities, though it is typically 50 times lower than the lowest calibration level C, but still detectable.

EN 14110 specifies a three-point linear calibration curve at 0.01%, 0.1% and 0.5% methanol by mass. Create calibration standards by adding 142 µL of methanol to 25 mL of blank biodiesel matrix. Label this standard dilution as Calibration A at a concentration of 0.5% mass methanol. Add 5 mL of dilution A to 20 mL of blank biodiesel matrix. Label this standard dilution as Calibration B at a concentration of 0.1% mass methanol. Add 1 mL of dilution B to 9 mL of blank biodiesel matrix. Label this standard dilution as Calibration C at a concentration of 0.01% mass methanol.

*Table 1.* Instrumental Conditions for Both the Standard and Modified EN 14110 Method.

| **GC Conditions** | **EN 14110** | **Modified EN 14110** |
|---|---|---|
| Oven: | 100 ˚C Isothermal | 50 ˚C Isothermal |
| Cap Injector: | 110 ˚C | 140 ˚C |
| Split: | off | 5 mL/min |
| Carrier Pressure: | off | 12 psig |
| FID Temperature: | 240 ˚C | 240 ˚C |
| FID Range/Attenuation: | 1/-2 | 1/-2 |
| **Headspace Conditions** | | |
| Oven: | 80 ˚C | 80 ˚C |
| Needle: | 90 ˚C | 105 ˚C |
| Transfer: | 110 ˚C | 120 ˚C |
| Thermostat: | 45 min | 10 min |
| Pressurize: | 2.0 min | 1.0 min |
| Inject: | 0.02 min | 0.04 min |
| Withdrawal Time: | 0.5 min | 0.5 min |
| GC Cycle Time: | 7.5 min | 5.0 min |
| Headspace Mode: | constant | constant |
| Injection Mode: | time | time |
| Column Pressure: | 20 psig | 17 psig |

**Sample preparation:** To follow the method presented in EN 14110, measure 5 g of each calibration standard into individual 22-mL headspace vials. Cap vials securely. Measure 5 g of each sample into headspace vials and cap securely. When using automated headspace, internal standard is optional but recommended, as it provides data to verify the quality and precision of the sample pressurization and injection.

To follow the modified method presented here, measure 250 µL of each calibration standard into individual 22-mL headspace vials with a positive displacement pipette. Cap vials securely. Likewise, measure analytical samples adding 250-µL B100 biodiesel into headspace vials and cap securely. Sample weights are not necessary when using a positive displacement pipette. Calibration and sample aliquot can be done by volume with a positive displacement pipette adding to the increased speed and simplicity of the overall method. Biodiesel samples are too viscous to measure with replaceable tipped pipettes.

## Results

The analysis of methanol in biodiesel with automated headspace GC-FID is a simple and accurate technique. The chromatographic data is very easy to interpret; resulting in a very simple chromatogram. The simplicity is a result of the non-volatile matrix (97% FAME by definition) with only a few volatile alcohols added during processing. The significant peaks in the chromatogram will be methanol and 2-propanol, if an internal standard is used.

Figure 1 (Page 3) demonstrates the analysis of methanol in biodiesel with 2-propanol as internal standard, a 0.5% weight standard and two sample analyses are also shown. The two samples pictured are a biodiesel: in one sample, the methanol was effectively removed, and in a second sample, the methanol was not removed completely. The large methanol peak is obvious in both the standard and the second sample.

In all three chromatograms in Figure 1, you see a consistent peak for 2-propanol, the internal-standard. In this case, the internal-standard was not used for calibration, rather as a measure of the quality of the headspace injection.

If the vial was improperly crimped or another type of systematic error occurred, the internal-standard area would change, providing the analyst with an indication of the problem. Consistent internal-standard area will improve confidence in the analytical results.

A three-point calibration was run using both analytical methods. The calibration demonstrated a linear response with both curves having $r^2$ values greater than 0.999 across the calibration range of 0.01% through 0.5%. Additional precision data was generated on each method with the traditional EN 14110 approach, generating approximately 5% RSD over 5 injections and the modified approach, generating less than 2% RSD over 5 injections.

## Conclusion

Demonstrated here is the analysis of methanol in B100 biodiesel. Automated headspace sample introduction is a simple, fast and clean technique. The non-volatile matrix is never in contact with the analytical system, eliminating associated maintenance. The automated system provides the laboratory with consistent, high-precision results.

The data shown was generated using both the traditional EN 14110 method and a modified method to improve the speed and precision of the analysis. EN 14110 methodology generated acceptable precision and outstanding linearity, with a 45-minute equilibration time and 7.5-minute injection-to-injection time. The modified methodology with a 250-µL sample volume and 10-minute equilibration time exhibited exceptional linearity and precision with a 5-minute injection-to-injection time.

## References


1. ASTM D6751-07a: Standard Specification for Biodiesel Fuel Blend Stock (B100) for Middle Distillate Fuels.
2. EN 14110: Fatty Acid Methyl Ester (FAME) Determination of Methanol.


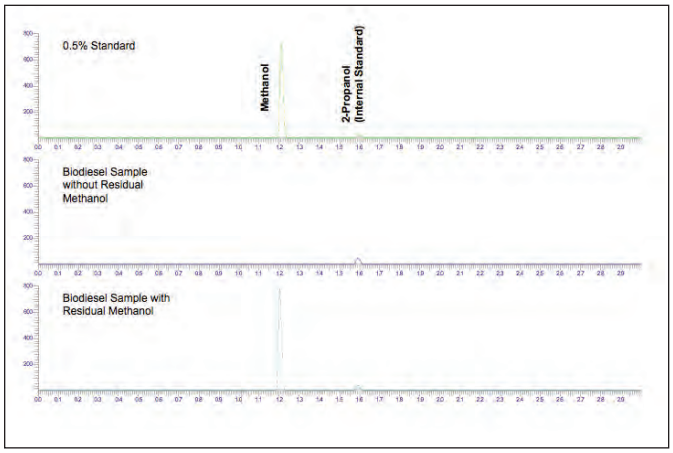


*Figure 1.* Chromatogram of the analysis of methanol in B100 biodiesel, following EN 14110 methodology.

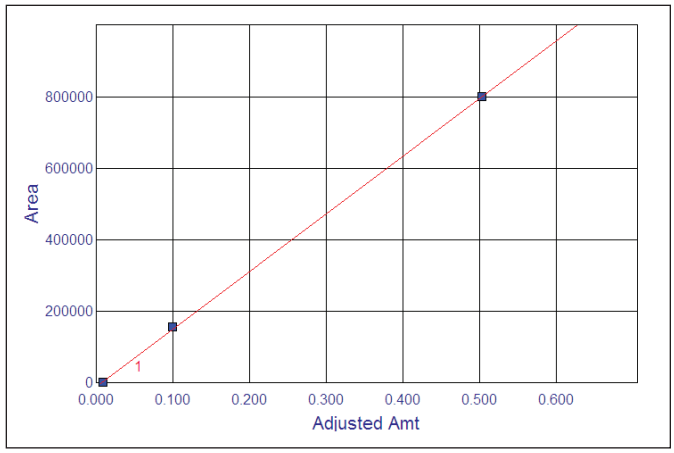


*Figure 2.* The calibration plot of a curve prepared with 5 g sample volume with linearity of 0.9999.


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008371_01

# Free and Total Glycerol in B100 Biodiesel by Gas Chromatography According to Methods EN 14105 and ASTM D6584

## Introduction

With today's increasing concern for the environment and the depletion of fossil fuel resources comes a greater awareness for alternative fuels, especially for biofuels. One of the more common biofuels is biodiesel, which is a renewable fuel from natural oils such as soybean oil, rapeseed oil or animal fats; it is a substitute for petroleum-diesel fuel.

Biodiesel consists of fatty acid alkyl esters produced by the transesterification reaction of vegetable oils and animal fats (Figure 1). When methanol is used for the transesterification reaction, fatty acid methyl esters (FAME) are formed. In addition to being a renewable fuel, biodiesel is also non-flammable, biodegradable and non-toxic, so it greatly reduces many environmental and transportation risks inherent to petroleum-based fuels.

To ensure high quality, criteria are set for many different properties of biodiesel – these criteria are specified in EN 14214 and ASTM D6751-07a. The most important criterion for a good-quality biodiesel is the completion of the transesterification reaction. Considering the short introduction to this reaction, it is easy to see why.

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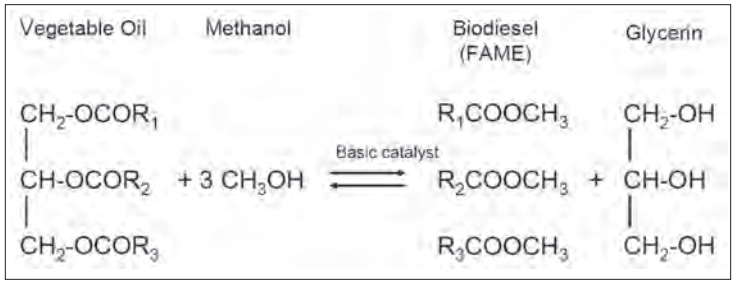


*Figure 1.* Illustration of the transesterification reaction of triglycerides to fatty acid methyl esters.



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When the triglycerides react with methanol, first, the corresponding diglycerides are formed together with the fatty acid methyl ester (FAME). The reaction continues and the diglycerides lose a second FAME and the monoglycerides are formed. Finally, a third FAME will be lost resulting in the free glycerol. Thus, incomplete reaction will give rise to un-reacted triglycerides from the parent vegetable oil or fat and the intermediates mono- and diglycerides. These are referred to as bound glycerol. Another contaminant found in the final biodiesel is remaining glycerol that has not been removed from the biodiesel during the water washing step. The latter is referred to as free glycerol. The sum of the bound and the free glycerol is referred to as total glycerol.

This paper will present the analysis of free and total glycerol by GC-FID following the methodology of both EN 14105 and ASTM D6584. Analysis of calibration standards and example biodiesel samples will be presented.

## Experimental

Sample preparation is a vital step in this analysis. The glycerol, mono-, di- and triglycerides must be derivitized to reduce their polarity and improve the thermal stability of the molecule. The derivatization technique used is silylation. The derivatization reagent to be used is MSTFA (N-methyl-N-(trimethylsilyl)trifluoroacetamide) – the reaction involves the replacement of the active hydrogen of the hydroxyl-group by a trimethylsilyl-group.

The calibration and internal standards are prepared in pyridine, according to the EN and ASTM procedure. Prepared calibration solutions are readily available (PerkinElmer Part No. N9331040) – this will simplify method setup, reduce preparation time, minimize the possibility of human error and eliminate the need to prepare dilutions.

The derivatization procedure for both standards and samples is identical. Weigh approximately 100 mg of sample or standard into a vial and record the actual weight. Internal standards are added according to the EN or ASTM specification, and 100 µL MSTFA (derivatization reagent) is finally added. The samples and standards are allowed to stand for 20 min at room temperature to allow the derivatization reaction to complete. Following derivatization, heptane is added and the vial is capped and shaken. The standards and samples are now ready for analysis.

Equally important to the success of this analysis are the instrumental conditions, supplies and the gas chromatograph (GC) configuration. As is clear from the previous discussion, the components analyzed are not the most favorable for gas chromatography. The high-boiling and thermally-labile compounds require a tightly-controlled injection technique. In GC, the most suitable injection technique to achieve a reproducible and controlled injection is the cool on-column injection. The instrumentation used in this paper is the PerkinElmer® Clarus® 600 GC fitted with the programmable on-column injector.

The analytical column used in this application must meet two major requirements:

1. The internal diameter of the column needs to be sufficiently wide to allow on-column injection
2. The column must withstand high oven temperatures

*Table 1.* Detailed Instrument Conditions.

| **Sample Introduction** | **PSS Injector** |
|---|---|
| Inlet Program Initial Temperature | 60 °C |
| Hold Time 1 | 1.00 min |
| Ramp 1 | 15 °C/min |
| Inlet Program Intermediate Temperature | 300 °C |
| Hold Time 2 | 0.00 min |
| Ramp 2 | 30 °C/min |
| Inlet Program Final Temperature | 380 °C |
| Column Flow | 3 mL/min |
| Injection Volume | 1 µL |
| **Gas Chromatograph** | **PerkinElmer Clarus 600 GC** |
| Oven Program Initial Temperature | 50 °C |
| Hold Time 1 | 1.00 min |
| Ramp 1 | 15 °C/min |
| Oven Program Temperature 2 | 180 °C |
| Hold Time 2 | 0.00 min |
| Ramp 2 | 7 °C/min |
| Oven Program Temperature 3 | 230 °C |
| Hold Time 3 | 0.00 min |
| Ramp 3 | 10 °C/min |
| Oven Program Final Temperature | 370 °C |
| Hold Time 4 | 5.00 min |
| Equilibration Time | 0.0 min |
| Column | Elite-Biodiesel M, 14 m x 530 µm x 0.16 µm film |
| Pre-column | Built-in 2 m Integra-Gap |
| Carrier Gas | Helium |
| FID Temperature | 380 °C |
| H2 flow | 45 mL/min |
| Air flow | 450 mL/min |
| Range | 1 |
| Attenuation | -5 |

You can achieve this 2 ways: a 0.32 mm i.d. fused silica analytical column butt connected to a 0.53 mm i.d. guard-column; or a metal analytical column with 0.53 mm i.d. and integrated guard column. The second option, which is used here, is preferable. The metal column eliminates the physical connection between the analytical column and the guard-column, reducing leaks and breakage. Additionally, the metal capillary column withstands higher oven temperatures, offering a more robust and reliable long-term solution.

## Results

The GC analysis of free glycerol, internal standards, mono-, di- and triglycerides identifies each analyte by its retention time. The retention time is determined by the analysis of a known reference standard. Reference standards are also used to generate a calibration curve, which relates FID response to % weight in the samples. The quantification of glycerol, mono-, di- and triglycerides requires a four-level calibration curve for EN 14105 and a five-level calibration curve for ASTM D6584. An internal-standard calibration is required by both methods.

*Table 2.* Summary of Calibration Results for Free and Total Glycerol Following Both EN and ASTM Methodology.

| | Calibration Summary | |
|---|---|---|
| | Linearity ($R^2$) EN 14110 (4-Point Calibration) | Linearity ($R^2$) ASTM D6584 (5-Point Calibration) |
| Glycerol | 0.9999 | 0.9999 |
| Total Monoglycerides | 0.9999 | 0.9984 |
| Total Diglycerides | 0.9999 | 0.9987 |
| Total Triglycerides | 0.9985 | 0.9945 |

In this paper, calibration curves presented for both EN 14105 and ASTM D6584 (Table 2) demonstrate excellent linearity, $R^2 > 0.99$ for each analyte. Glycerol is quantified as a single peak, with butanetriol as the internal standard. The monoglycerides are calibrated as a timed group of the 5 monoglycerides, with tricaprin as the internal standard. Total monoglycerides cannot be calculated as a summed time group due to co-elution of the $C_{24}$ ester. The di- and triglycerides are quantified as timed groups, also with tricaprin as the internal standard. The calibration plots are saved in the data processing method of TotalChrom® Chromatography Data Systems (CDS) – a calibration plot is presented in Figure 2.

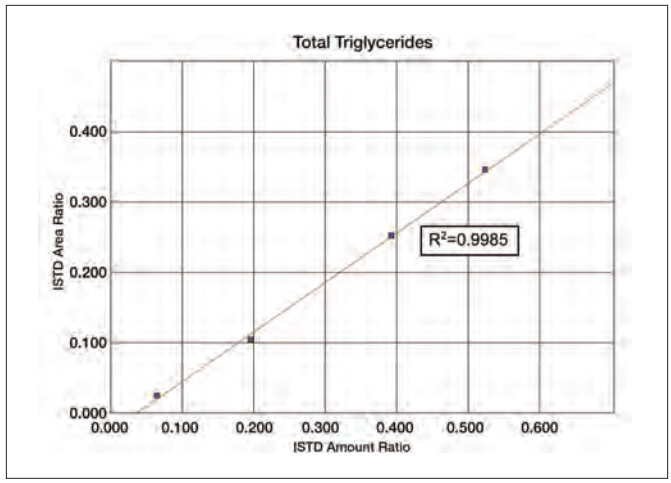


*Figure 2.* Example calibration plot for total triglycerides following EN 14105.

An example chromatogram of a calibration standard is pictured in Figure 3 (Page 4): glycerol and butanetriol are the first two peaks (callout box 1); following that, monoolein, tricaprin (internal standard), two diglyceride peaks and a single triglyceride peak (callout box 2) elute. The calibration standards only include 1 of the 5 monoglycerides; EN 14105 requires that a mixture of all 5 monoglycerides be analyzed to determine the retention time of each experimentally, for positive identification. The ASTM methodology uses relative retention-time data to identify each monoglyceride.

This paper includes the analysis of 2 different biodiesel samples – a washed soy biodiesel and an unwashed used vegetable-oil biodiesel (Figures 4 and 5 respectively). In the washed soy biodiesel sample (Figure 4 - Page 4), the internal standard peaks are clearly visible, along with a cluster of peaks for the FAME content of the sample (9.5-15 min). There are no visible peaks for either free or bound glycerol. This is indicative of a complete reaction and washing. Insufficient washing would demonstrate increased glycerol content. An incomplete transesterification would result in mono- di- and triglycerides detection. In this case, the production facility has a controlled process and a final product which will meet both ASTM and EN standards.

A biodiesel sample without complete transesterification and washing is pictured in Figure 5 (Page 5). The result is large peaks corresponding to glycerol (callout box 1), monoglycerides (callout box 2), di- and triglycerides (callout box 3). There is also an increase in the presence of matrix peaks (broad peaks around 20 and 22 minutes).

In this case, the production facility needs to modify their procedures and improve the washing step of the process. This will complete the reaction and remove residual glycerol. It would be expected that this sample will have elevated methanol and potassium hydroxide levels, also as a result of incomplete washing.

The final step in the free and bound glycerol analysis is the reporting of % weight results. EN 14105 and ASTM D6584 present detailed calculations for this determination. The PerkinElmer TotalChrom CDS, used here, will perform the calculations and report the results. An example free-and-bound-glycerol report is pictured in Figure 6 (Page 5).

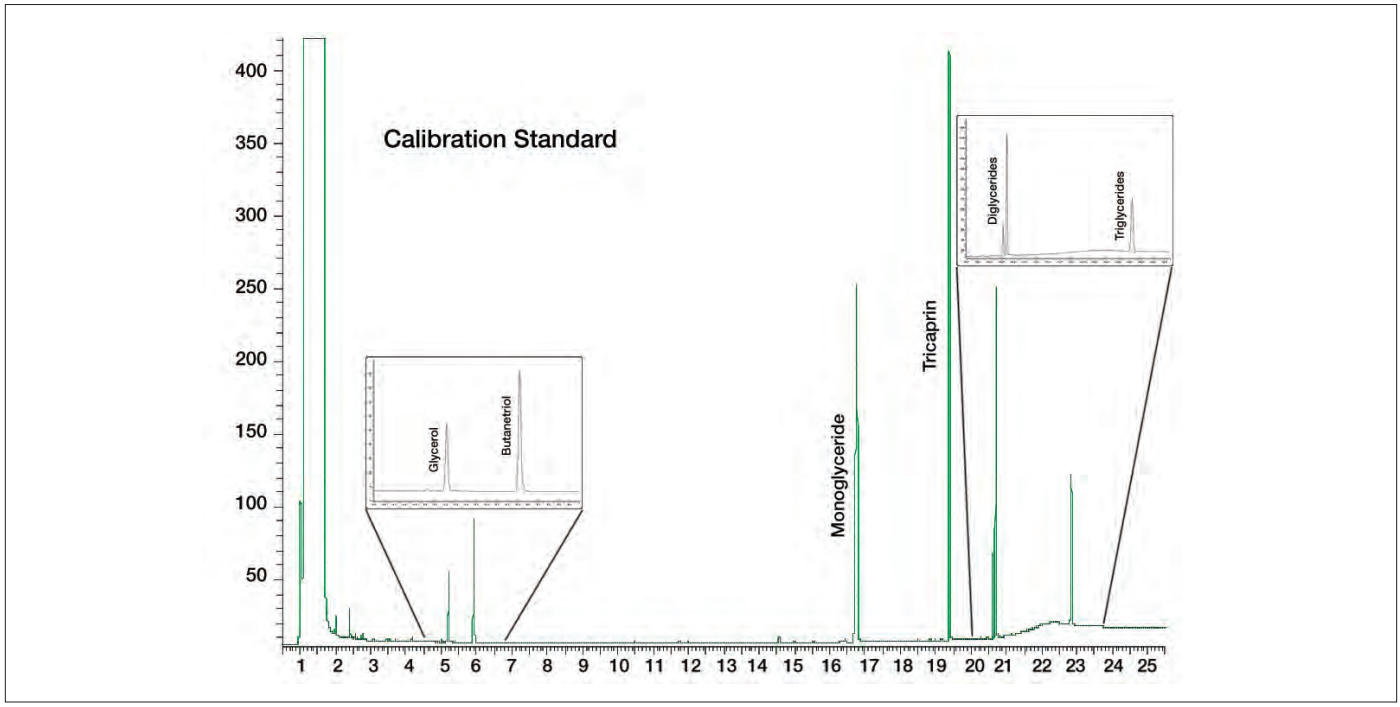


*Figure 3.* Free and total glycerol calibration standard.

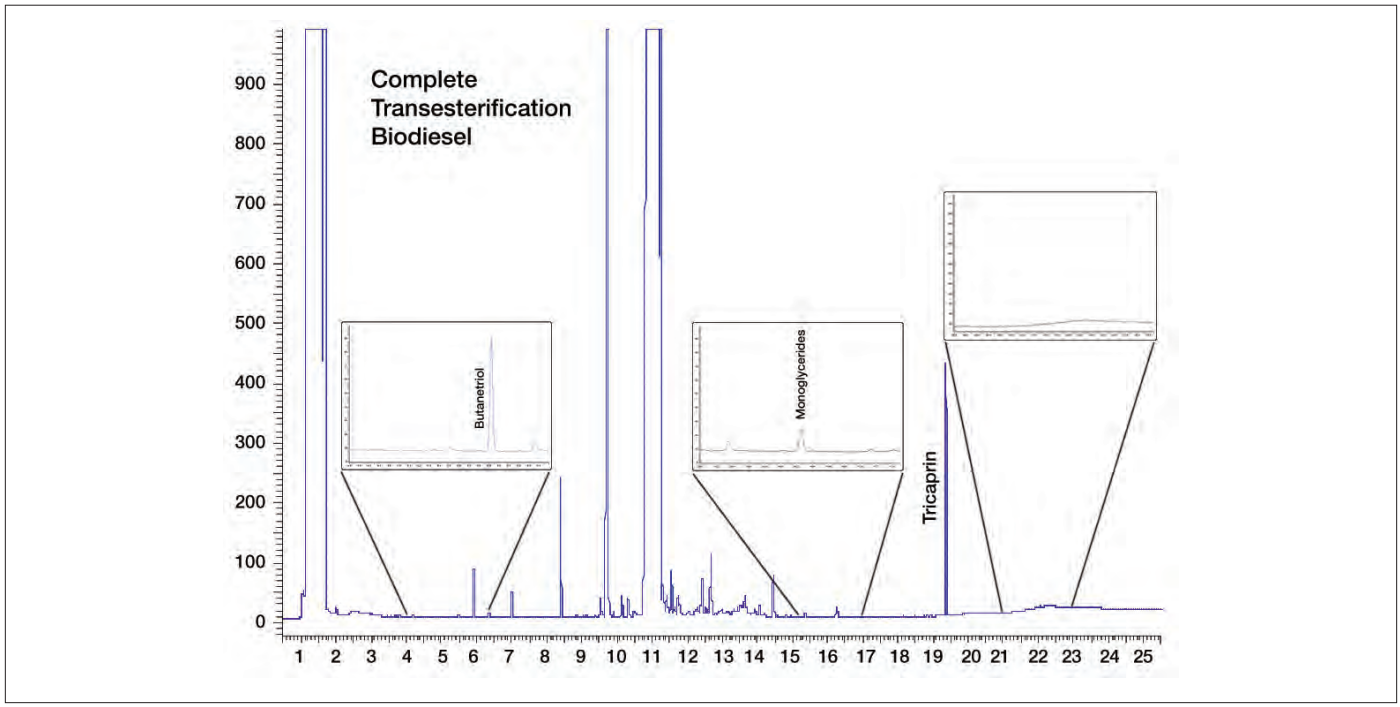


*Figure 4.* Sample biodiesel in which the transesterification reaction was completed.

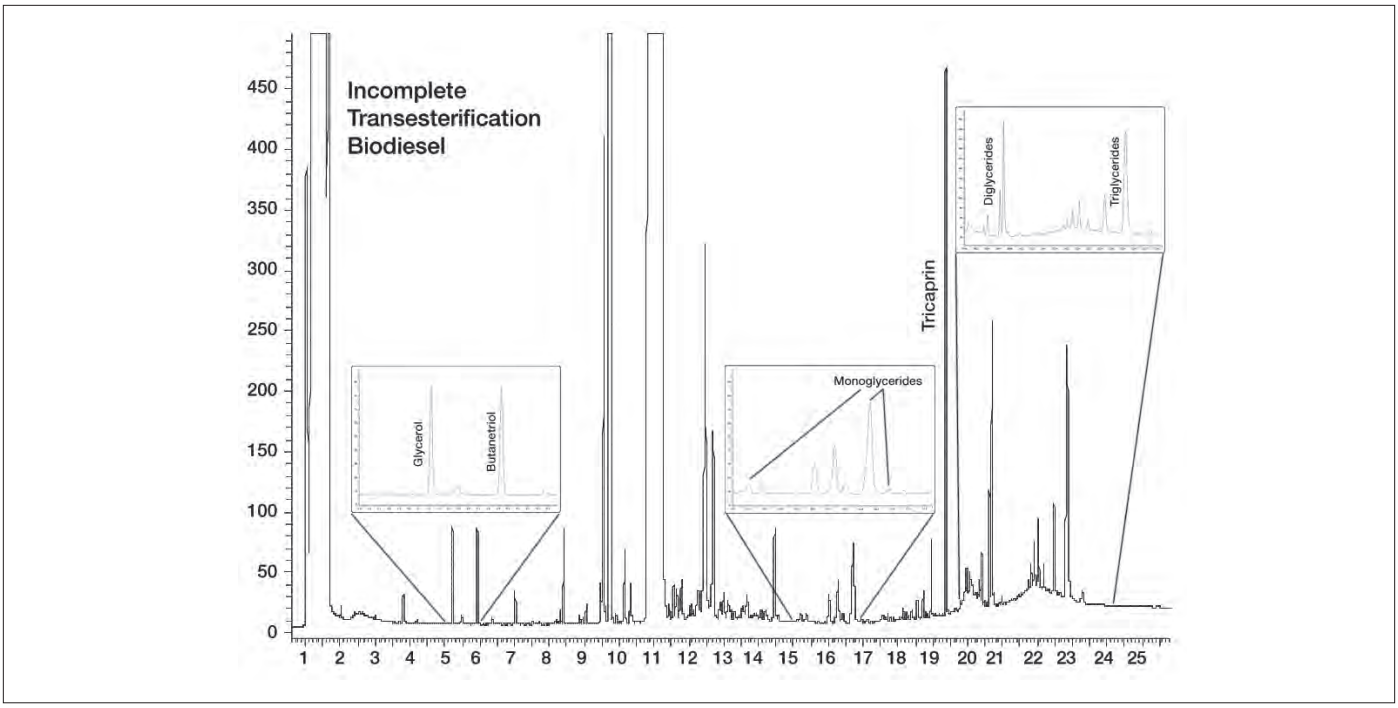


*Figure 5.* Sample biodiesel in which the transesterification reaction is incomplete.

## Conclusion

As the distribution of biofuels, particularly biodiesel, expands, the focus on quality becomes more important. Biodiesel is a substitute for petroleum-based diesel fuel – however, the methods to determine fuel quality are different than the traditional methods for the analysis of petroleum fuels. In this paper, the analysis of free and bound glycerol by EN 14105 and ASTM D6584 was presented. The Clarus GC configured with an on-column injector, flame ionization detector and metal biodiesel capillary column provided the platform for analysis. The system calibration demonstrated linear response for glycerol, mono-, di- and triglycerides. The automation of TotalChrom CDS simplified the calculation and result reporting, delivering a simple report with the percent weight of free and bound glycerol.

| Component Name | Time [min] | Area [uV*sec] | Amt Mass % | Total Mass % |
|---|---|---|---|---|
| Glycerol | 5.632 | 5638.38 | 0.00 | 0.00 |
| Butanetriol | 6.372 | 145217.36 | – | – |
| Total Monos | 17.643 | 385800.17 | 0.60 | 0.16 |
| Tricaprin | 19.893 | 441384.15 | – | – |
| Diglycerides | 21.125 | 43656.13 | 0.08 | 0.01 |
| Triglycerides | 24.500 | 515.33 | 0.01 | 0.00 |
| | | | | 0.17 |

*Figure 6.* Example TotalChrom report for free and bound glycerol.

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008387_01

# Fatty Acid Methyl Esters in B100 Biodiesel by Gas Chromatography (Modified EN 14103)

## Introduction

The production and consumption of biofuels continues to increase as more attention is paid to the environment and the depletion of fossil-fuel resources. Biodiesel, a fuel from natural oils such as soybean oil, rapeseed oil or animal fats, is a substitute for petroleum-diesel fuel. The quality criteria for the production of biodiesel are specified in EN 14214.

Within EN 14214, method EN 14103 specifies the fatty acid methyl ester (FAME) and linolenic acid methyl ester content (Figure 1), which is used to profile the vegetable or animal oil feedstock used in biodiesel production. EN 14103 calls for calibration of all FAME components by relative response to a single compound, methyl heptadecanoate. This requires the measurement of accurate weights for each sample and the addition of an internal standard. The range of FAMEs for which the method is intended lies between $C_{14:0}$ and $C_{24:1}$.

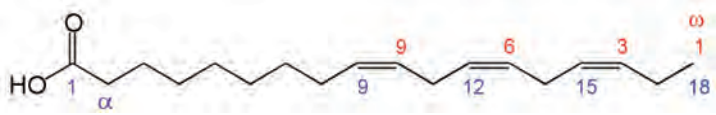


*Figure 1.* Linolenic acid.

This application note will discuss the analysis according to method EN 14103. In addition to the methodology specified in EN 14103, a simpler and more accurate method will be presented. The modified method uses commercially-available calibration and test mixtures for precise peak identification and quantitative accuracy, while streamlining the sample preparation and calculations. Reporting is based on area % of all components after the solvent – as a result, the sample weight does not impact the calculations.

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## Experimental

The FAME analysis is carried out with a split injection onto an analytical column with a polar stationary phase and an FID detector. The configuration used here is the PerkinElmer® Clarus® 600 Gas Chromatograph (GC), fitted with a capillary split/splitless injector and FID. It is very important to choose the appropriate liner; otherwise, the response, reproducibility and resolution of the analysis will be compromised. Fatty acid methyl esters are known to be active and thermally-labile components. Incorrect injection conditions and liner choice will result in a non-linear response; the late-eluting FAMEs (longer carbon chains) show a lower response than the early-eluting FAMEs. This problem is overcome by packing the inlet liner with glass wool, greatly improving uniformity and reproducibility over the boiling-point range. Deactivated liners (with wool) are available. The surface of the liner is deactivated to minimize bleed and to enhance the inertness of the liner.

The analytical column used in this work is the PerkinElmer Elite-Famewax column (Crossbond® polyethylene glycol), which demonstrates good resolution and peak shape (Figure 2). Table 1 provides an overview of all the instrument parameters.

*Table 1.* Instrument Parameters EN 14103.

| **Gas Chromatograph:** | **PerkinElmer Clarus 600 GC** |
|---|---|
| Inlet Temperature: | 250 °C |
| Column Flow: | 1 mL/min |
| Split Flow: | 50 mL/min |
| Injection Volume: | 0.5 µL |
| Oven Program Initial Temp: | 210 °C |
| Hold Time 1: | 13.00 min |
| Ramp 1: | 5 °C/min |
| Oven Program Final Temp: | 230 °C |
| Hold Time 2: | 15.00 min |
| Equilibration Time: | 0.0 min |
| Column: | Elite-Famewax, 30 m x 320 µm x 0.25 µm film |
| Carrier Gas: | Helium |
| FID Temperature: | 250 °C |
| $H_2$ Flow: | 45 mL/min |
| Air Flow: | 450 mL/min |
| Range: | 1 |
| Attenuation: | -5 |

In order to determine the retention times of the fatty acid methyl esters, a FAME standard needs to be run. These are available commercially either separately or as a standard reference mixture. The analysis of a commercial FAME standard is shown in Figure 2.

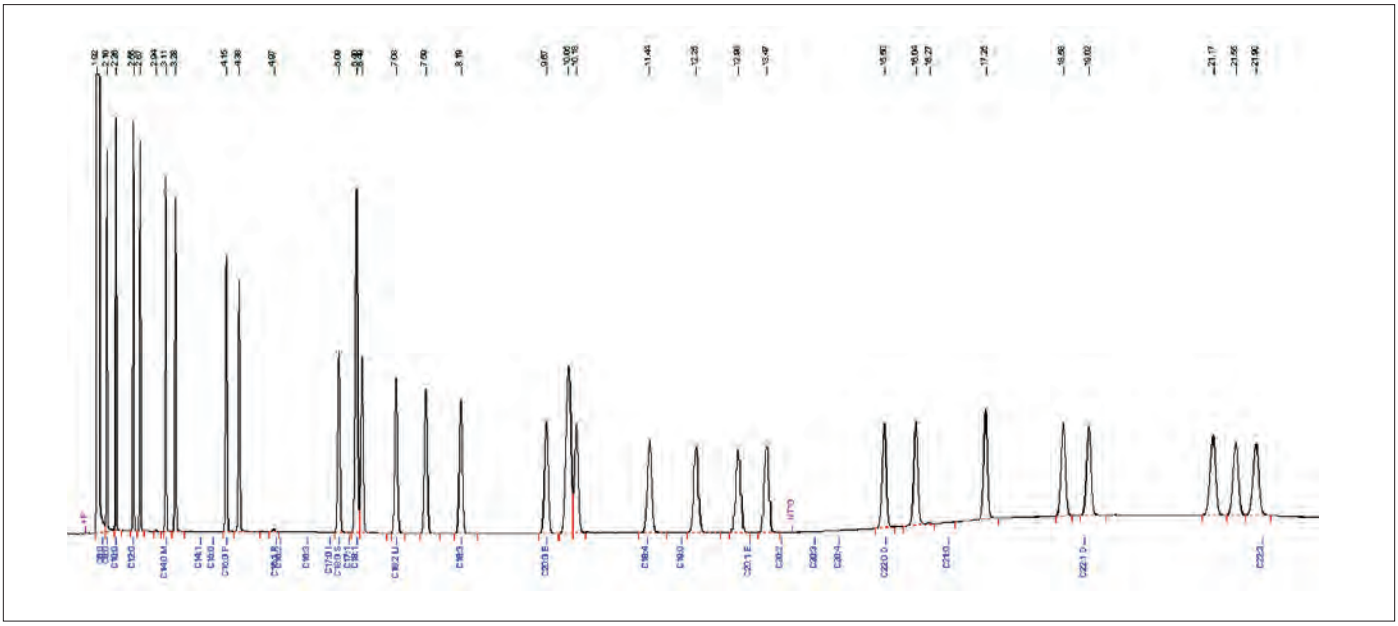

*Figure 2.* The analysis of a mixture of $C_{14:0}$-$C_{24:1}$ FAMEs.

The modified method of analysis uses a FAME mixture at known concentration and determines the response and retention time of each component experimentally. The reporting is then based on an area % rather than a mass %, simplifying the calculations. The calibration is verified by the analysis of a calibration-check standard – typically, this is commercial mix of FAMEs from a second source with a certificate of analysis. This standard would be analyzed after the calibration of the response. The results of the analysis are then compared with the certificate of analysis, verifying the quality of the calibration. The standard preparation for this technique consists of the dilution of the FAME standard into 4 mL of n-heptane. The sample preparation is also quite simple with 100 µL of biodiesel feedstock into 4 mL of n-heptane. In both cases, a 1-µL injection at a split of 50:1 is performed. The full instrument conditions are presented in Table 2.

*Table 2.* Modified Parameters for the Analysis of FAMEs in Biodiesel.

| **Gas Chromatograph:** | **PerkinElmer Clarus 600 GC** |
|---|---|
| Inlet Temperature: | 240 °C |
| Column Flow: | 2 mL/min |
| Split Flow: | 50 mL/min |
| Injection Volume: | 1 µL |
| Oven Program Initial Temp: | 195 °C |
| Hold Time 1: | 0 min |
| Ramp 1: | 5 °C/min |
| Oven Program Final Temp: | 240 °C |
| Hold Time 2: | 6 min |
| Column: | Carbowax 20 M, 30 m x 320 µm x 0.25 µm film |
| Carrier Gas: | Helium |
| FID Temperature: | 240 °C |
| $H_2$ Flow: | 45 mL/min |
| Air Flow: | 450 mL/min |

## Results

In EN 14103, the result for the fatty acid methyl ester content is expressed as a mass fraction in percent using methyl heptadecanoate ($C_{17}$) as the internal standard. Total FAME content should be greater than 90%. Linolenic acid ($C_{18:3}$) content should be greater than 1% and less than 15%. The following formula is used:

$$C = \frac{\Sigma A - A_{IS}}{A_{IS}} \times \frac{C_{IS} \times V_{IS}}{m} \times 100\%$$

**Where:**

$\Sigma A$ = total peak area $C_{14:0} - C_{24:1}$

$A_{IS}$ = internal standard (methyl heptadecanoate) peak area

$C_{IS}$ = concentration of the internal standard solution, in mg/mL

$V_{IS}$ = volume of the internal standard solution used, mL

m = mass of the sample, in mg

Linolenic acid methyl ester content is also expressed as a mass fraction in percent and methyl heptadecanoate ($C_{17}$) is used as the internal standard. The following formula applies:

$$L = \frac{A_L}{\Sigma A - A_{IS}} \times 100\%$$

**Where:**

$\Sigma A$ = total peak area $C_{14:0} - C_{24:1}$

$A_{IS}$ = internal standard (methyl heptadecanoate) peak area

$A_L$ = linolenic acid methyl ester peak area

The result of the mass fraction calculation is then used to calculate the sample's iodine value, which is the sum of the individual contributions of each methyl ester, obtained by multiplying the methyl ester percentage by its respective factor. The following formula applies:

***Iodine value = X g iodine / 100 g sample***

Table 3 (Page 4) shows an example calculation for the iodine factor.

*Table 3.* Iodine Factor Example.

| Methyl Ester of Following Acids in Sample (as example) | Amount in % Mass | Iodine Factor | Contribution |
|---|---|---|---|
| Myristic $C_{14:0}$ | 0.3 | 0 | 0.00 |
| Palmitic $C_{16:0}$ | 4.0 | 0 | 0.00 |
| Palmitoleic $C_{16:1}$ | 1.1 | 0.950 | 1.05 |
| Stearic $C_{18:0}$ | 2.0 | 0 | 0.00 |
| Oleic $C_{18:1}$ | 60.5 | 0.860 | 52.03 |
| Linoleic $C_{18:2}$ | 19.8 | 1.732 | 34.29 |
| Linolenic $C_{18:3}$ | 9.4 | 2.616 | 24.59 |
| Eicosanoic $C_{20:0}$ | 0.4 | 0 | 0.00 |
| Eicosenoic $C_{20:1}$ | 0.7 | 0.785 | 0.55 |
| Docosanoic $C_{22:0}$ | 0.7 | 0 | 0.00 |
| Docosenoic $C_{22:1}$ | 1.1 | 0.723 | 0.80 |
| | | **Calculated Iodine Value** | **113.3** |

## Conclusion

Method EN 14103 is used to determine the fatty acid methyl ester (FAME) between $C_{14:0}$ and $C_{24:1}$ and linolenic acid methyl ester content of oil feedstock used in biodiesel production. EN 14103 calls for calibration of all FAME components by relative response to a single compound – methyl heptadecanoate. This application note has demonstrated the analysis according to method EN 14103. Additional methodology presented a simple analysis using commercially-available calibration and test mixtures.

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008392_01

# Biodiesel IR Fatty Acid Methyl Ester (FAME) Analyzer

ecoanalytix™

INFRARED SPECTROSCOPY

PRODUCT NOTE

## Introduction

The potential of biofuels to provide an economical, clean-burning, and sustainable source of fuel, now and for the future, has led to a growing global commitment to their use.

The production and distribution of alternative fuels is strictly regulated by both national and international regulatory bodies. PerkinElmer®, via its EcoAnalytix™ initiative, provides a series of solutions comprised of analytical instrumentation, standard operating procedures, training and support to help meet such regulatory requirements.

## Biodiesel IR FAME Analyzer

Biodiesel, derived from vegetable feedstocks such as soybean and rapeseed or from animal fats, is a fuel commodity primarily used as a value-added blending component with diesel fuel. The term biodiesel describes a fuel of pure mono alkyl esters such as fatty acid methyl ester (FAME), and is designated B100. The term Bxx (e.g. B20, B30 etc.) is used to describe a blend of biodiesel with petroleum-based diesel fuel.

The international standard ASTM D7371-07 specifies a quality control method for the production and distribution of diesel and blended fuels containing FAME. The test method applies the use of a Fourier Transform Infrared (FT-IR) instrument with an Attenuated Total Reflectance (ATR) sampling accessory. The absorption spectra of samples where the percentage of FAME concentration is known are used to develop calibrations against which FAME concentrations of unknown samples can be confirmed.

## Key Benefits

- State-of-the-art FT-IR and sampling with built-in diagnostics and calibration functions to ensure the most accurate and reliable data
- ASTM D7371-07 calibrations and prediction protocol supplied so instrument is up and running in the shortest possible time
- Automated, single-click calculation protocol for easy, reproducible results
- On-demand output diagnostics give additional insights into the errors associated with the results to provide highest confidence in analysis
- Built-in automated system suitability feature for biodiesel enables system to be qualified prior to analysis using relevant test samples
- Flexible reporting for easy output to other reporting packages and control charting

The PerkinElmer Biodiesel IR FAME Analyzer comprises a Spectrum™ 100 FT-IR spectrometer with ATR accessory, and Spectrum Express™ software that is configured specifically for the ASTM D7371-07 methodology.

The Analyzer includes three (3) starter calibrations for FAME derived from soy oils covering the ranges 0-10%, 10-30% and 30-100% FAME. Spectrum QUANT+™ software is provided so calibrations can be validated independently and updated as necessary with customer samples. Validation is recommended so any differences between feedstocks (rapeseed, palm, sunflower seed, etc.) are modeled on the chemometric calibrations.

Spectrum Express software simplifies sample analysis by the use of Process Chains. With a single click, the percentage FAME concentrations are calculated and the results printed, individually saved to file as a report.

The Biodiesel IR FAME Analyzer is based on a high performance benchtop PerkinElmer Spectrum 100 Fourier Transform Infrared spectrometer (FT-IR). This instrument combines highest performance FT-IR with instrument control, biodiesel analysis and reporting software. The sampling interface is an extremely rugged diamond surface with highest resilience to damage due to sample abrasion and cleaning. Just a few drops of sample are required to cover the sampling crystal, and the data is collected and report generated in less than 1 minute.

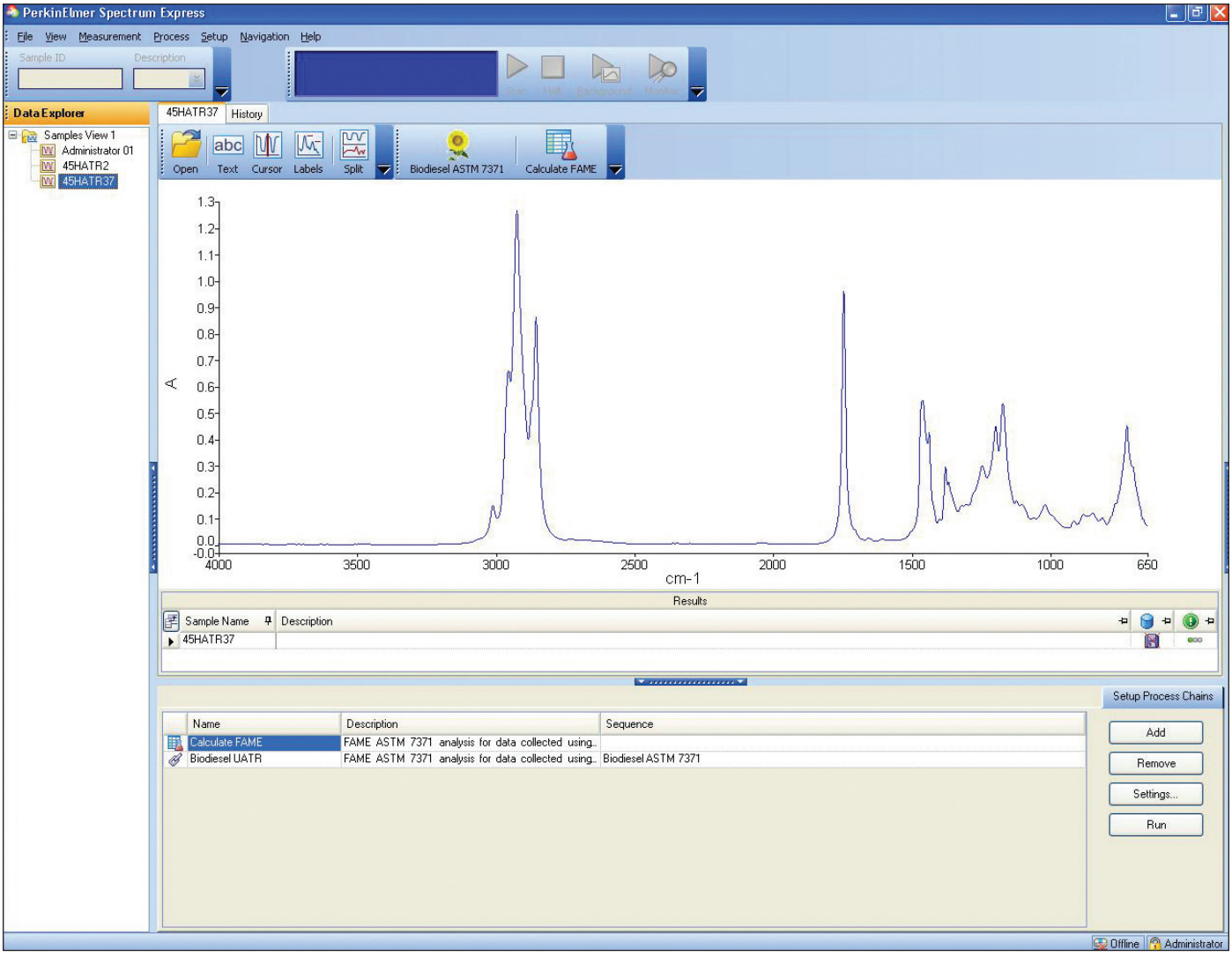
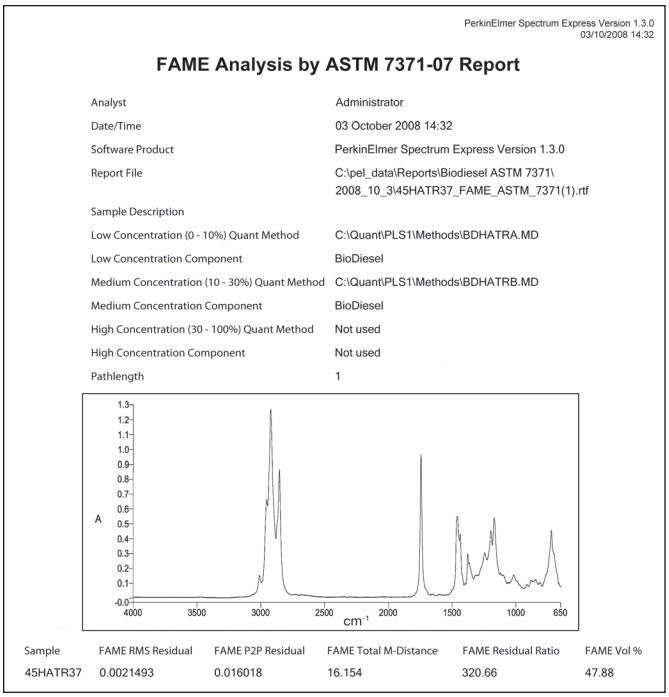



*Figure 1.* Spectrum Express biodiesel software and results table.



PerkinElmer Spectrum Express Version 1.3.0
03/10/2008 14:32

**FAME Analysis by ASTM 7371-07 Report**

| | |
|---|---|
| Analyst | Administrator |
| Date/Time | 03 October 2008 14:32 |
| Software Product | PerkinElmer Spectrum Express Version 1.3.0 |
| Report File | C:\pel_data\Reports\Biodiesel ASTM 7371\ 2008_10_3\45HATR37_FAME_ASTM_7371(1).rtf |
| Sample Description | |
| Low Concentration (0 - 10%) Quant Method | C:\Quant\PLS1\Methods\BDHATRA.MD |
| Low Concentration Component | BioDiesel |
| Medium Concentration (10 - 30%) Quant Method | C:\Quant\PLS1\Methods\BDHATRB.MD |
| Medium Concentration Component | BioDiesel |
| High Concentration (30 - 100%) Quant Method | Not used |
| High Concentration Component | Not used |
| Pathlength | 1 |



| Sample | FAME RMS Residual | FAME P2P Residual | FAME Total M-Distance | FAME Residual Ratio | FAME Vol % |
|---|---|---|---|---|---|
| 45HATR37 | 0.0021493 | 0.016018 | 16.154 | 320.66 | 47.88 |

*Figure 2.* ASTM D7371-07 report.

The software provides an unprecedented degree of user flexibility with the ability to configure the system to provide single push-button control over all the steps of the analytical procedure including specific system performance checks for biodiesel analysis, data collection, data analysis according to the ASTM D7371-07 protocol, and report generation.

Calculated concentrations can be processed further using the unique Process Chains facility in the software to provide customized results and multiple predictions output to charts for results trending and statistical analysis. To further simplify operation and assist with good measurement practice, the user interface is highly configurable to allow just those essential functions to be available to the analyst, and a number of in-built intelligence features and diagnostics such as Quality Checks provide the operator with visual warnings of potential data problems which could influence results.

## Conclusion

With proven leadership in product innovation, including the introduction of the first commercial instruments in Infrared Spectroscopy (IR), Gas Chromatography (GC), and Graphite Furnace Atomic Absorption (GFAA) Spectroscopy, PerkinElmer continues to make advances in analytical solutions, including EcoAnalytix complete testing systems for the environment, food safety and renewable energy industries. Combining instruments, standard operating procedures, training and support, EcoAnalytix analyzers are designed to help these industries meet regulatory requirements.

***For more information, visit www.perkinelmer.com/biofuels***

## Ordering information

| Product | Part No. |
|---|---|
| Biodiesel IR FAME Analyzer UATR Sp100 LiTa | L125000P |

The Biodiesel IR FAME Analyzer is a total solution for FAME analysis. A number of pre-configurations is available. For routine FAME determination, we recommend the system equipped with universal ATR sampling. For further information on this and other configurations, contact your local PerkinElmer representative.

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008367_01

APPLICATION NOTE

FT-IR SPECTROSCOPY

# Biodiesel Concentration Measurements Using Attenuated Total Reflectance (ATR)

## Introduction

Reducing our dependence on fossil fuels and our reliance on oil and petroleum supplies are worldwide issues. Many see the increasing use of biodiesel fuel as a key initiative to meet these global needs. However, the move to include proportions of biodiesel in everyday fuel has created a host of unresolved issues for both engine manufacturers and diesel consumers. Uppermost among these are questions concerning the concentration of the biofuel (Fatty Acid, Methyl Ester – FAME) and its quality. This application note describes how infrared transmission measurements can be used to address the concentration measurements.

Biodiesel fuels are often blended compositions of diesel fuel and esterified soybean oils, rapeseed oils, or other potential vegetable oils as well as fats. The physical and combustion properties of these biofuels have allowed them to achieve similar performance to diesel fuel. However, there are several characteristics (including cetane number, oxidation stability, and corrosion potential) that are of concern. These differences, especially the cetane reduction, require that adequate control of the biofuel concentration be implemented.

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In addition, there are now tax incentives available in some parts of the world for the use of biodiesel. For example, in the USA this tax credit is presently in the form of a $0.01 per FAME-% per gallon of fuel used. Therefore, the difference between 19% or 20% FAME in diesel fuel can result in a considerable tax value. A recent investigation of commercially available biofuel blends identified that 18 out of 50 splash-blended samples were not the specified 20% FAME value (1). It can be seen that there are financial justifications for an accurate biofuel concentration determination and characterization.

This work was performed using the Spectrum™ 100 FT-IR spectrometer. The complete system consists of three elements:

- Spectrum 100 FT-IR spectrometer with high sensitivity, sampling speed, and stability.
- Universal ATR (UATR) plug-and-go accessory with integrated diagnostics; using a 9-bounce, liquid sampling top-plate.
- PerkinElmer® infrared quantitative software suite allowing analysis by various methodologies from Beer's Law concentration calculations using peak height measurements through to full Principal Component Analysis (PCA) chemometric analysis.

## EN method using Beer's Law

One of the few defined methods for measuring the concentration of FAME is EN 14078 (July 2004) – "Liquid petroleum products – Determination of fatty acid methyl esters (FAME) in middle distillates - Infrared spectroscopy method" (2).

The principle of the EN method is the application of a simple quantitative model of FAME content using the 1745 $cm^{-1}$ carbonyl absorbance. When using the EN methods, samples are diluted in cyclohexane to a final analysis concentration of 0-1.14% FAME. This produces a carbonyl peak intensity range between 0.1-1.1 Abs, using a 0.5-mm cell pathlength. The peak height of the carbonyl band at or near 1745 $cm^{-1}$ is measured to a baseline drawn between 1820 and 1670 $cm^{-1}$. This peak height is used with a Beer's Law plot (absorbance versus concentration) to develop the calibration curve used for calculating the unknown concentrations.

While it is possible to achieve good concentration measurement, the disadvantages of this method are the need for sample dilution and the inability of the simple methodology to cope with variances in the source of the biofuel. An improved solution, like ASTM D7371, utilizes ATR to reduce the effective pathlength and avoid the sample dilution errors. As there is a potentially increasing variance in chemistry of the sources of the FAME (namely soybean, rapeseed, or yellow-grease), peak area is proposed as a preferred calculation technique.

## Beer's Law method

This method used the 9-bounce UATR to analyze the FAME content of a biodiesel sample. The method that was employed in this study included:

- Peak area calculation – range: 1820-1670 $cm^{-1}$ with baseline set at the same range.
- No dilution – samples were not diluted to allow determination usable range

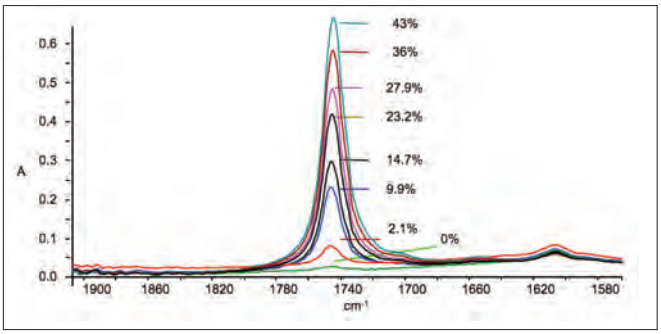


*Figure 1.* FT-IR spectra of varying FAME concentrations in diesel fuel.

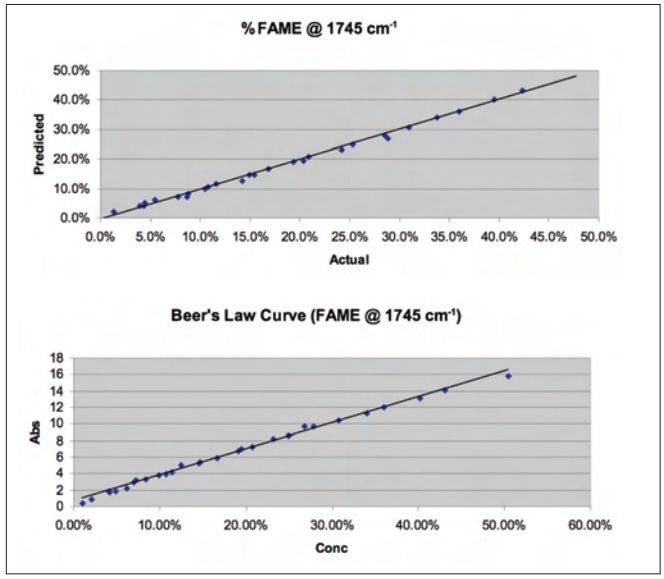


*Figure 2.* Beer's Law calibration method for 1745 $cm^{-1}$ peak.

Since one is determining a peak area within the Beer's Law experiment to be a valid method, the peak maximum cannot exceed the detector linearity range. Figure 1 shows the spectra for 1900 to 1600 $cm^{-1}$ of a series of varying concentration biofuel (FAME).

Figure 1 demonstrates that a Beer's Law curve is possible for this spectral region to above 43% FAME. A Beer's Law method was developed for these samples and is shown in Figure 2.

This Beer's Law method took a baseline as defined in the EN 14078 method at 1820-1670 $cm^{-1}$ and a peak area in the same range. The sample concentration range for this method was B0 to B43 (0% to 43% FAME). The method produced a linear curve with a correlation coefficient of 0.995. Calculating the concentration of the standards by the method yielded a Pearson's correlation of 0.998 and a standard error of prediction (SEP) of 1.2%. These results show that this method is somewhat marginal for the quantitation of FAME.

## Chemometric method

Chemometrics application using Principal Component Regression (PCR) was employed to determine the FAME concentrations between 0 and 50% FAME. The model employed as much of the entire spectrum (4000-650 $cm^{-1}$) as possible. Since the sampling technique used for these analyses was the 9-bounce UATR, there is a region of high absorbance in the spectrum that is associated with the diamond (2495-1822 $cm^{-1}$). This region needed to be blanked from the PCR model as it just adds to the noise of the model. This method utilized only one principal component. The spectrum of this principal component, referred as the Regression Spectrum, is shown in Figure 3. It shows that there are features of both the FAME (1745 and 1170 $cm^{-1}$) and the diesel fuel (3012, 1605 and 810 $cm^{-1}$) contributing to the analyses.

The statistics of this model showed a correlation coefficient of 0.9996 and a standard error of prediction (SEP) of 0.4%. The actual against predicted results for this model is shown in Figure 4; also indicating a good prediction model.

This chemometric approach to the analyses is much better than the Beer's Law method. Building a PCR model can be more difficult than Beer's Law; however, PCR does allow better statistics to tell if the results are consistent with the development standards.

## Conclusion

We have shown how the ATR technique can be used to address FAME concentration measurements. The preferred methodology uses chemometrics to analyze the whole spectrum, achieving a standard error of prediction of 0.4%. This compares well with the concentration measurement of FAME in a typical "splash blend" operation, where an error of 0.5% is usually acceptable.

A key advantage of using an ATR sampling method is speed and simplicity. This can really help in laboratories where multiple analyses are routinely performed. The choice of either Beer's Law or chemometrics will be determined by the particular situation. The Beer's Law approach benefits from being

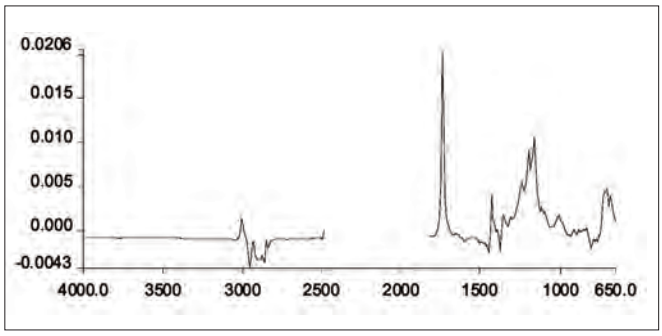


*Figure 3.* Biodiesel FAME concentration – PCR regression spectrum.

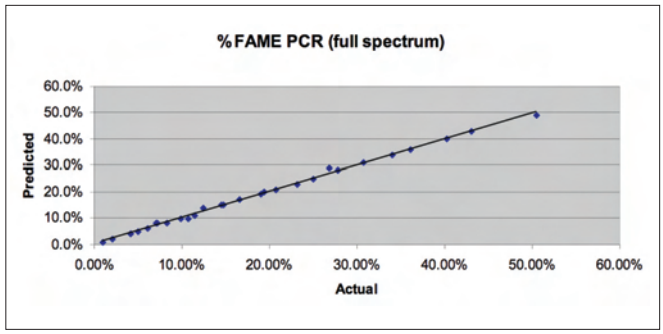


*Figure 4.* PCR calibration method.

simpler but is only recommended for situations where there is a low throughput of samples and the accuracy requirements aren't high. The chemometrics approach has the advantage of being more robust with respect to known constituents in the blend, better handling of interferents, and reduced effect from noise contributions. Overall, PCR offers far higher confidence in the quantitative prediction than is found using the Beer's Law methods.

## References


1. NREL Technical Report TP-540-38836, *Survey of the Quality and Stability of Biodiesel and Biodiesel Blends in the United States in 2004;* R.L McCormick, T.L. Alleman, M. Ratcliff, L. Moens, and R. Lawrence, (October 2005).
2. EN 14078 *Liquid Petroleum Products – Determination of Fatty Acid Methyl Esters (FAME) in Middle Distillates – Infrared Spectroscopy Method* (July 2004).
3. ASTM D7371 Standard Test Method for "Determination of Biodiesel (Fatty Acid Methyl Esters) Content in Diesel Fuel Oil Using Mid Infrared Spectroscopy".


## Acknowledgements


The experiments and analysis in this work were performed for PerkinElmer by independent oil analysis specialist David L. Wooton, Ph.D., of Wooton-Consulting, Beaverdam, VA, USA.



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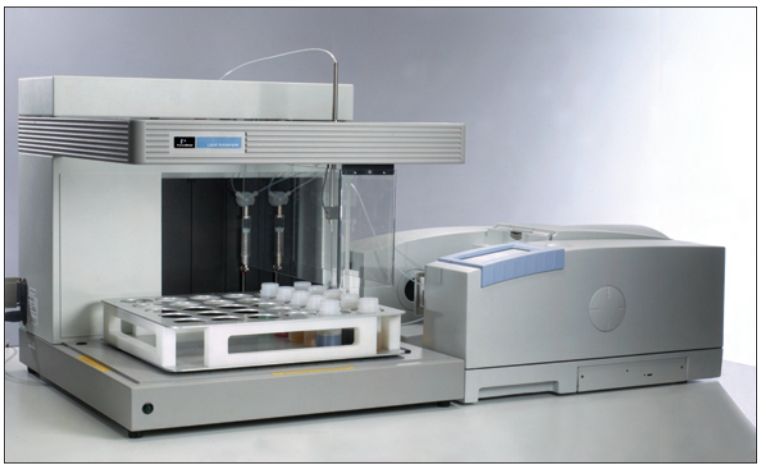
APPLICATION NOTE

FT-IR SPECTROSCOPY

# Biodiesel Concentration Measurements Using Spectrum OilExpress



## Introduction

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Biodiesel fuels are often blended compositions of diesel fuel and esterified soybean oils, rapeseed oils, or other potential vegetable oils, as well as fats. The physical and combustion properties of these biofuels have allowed them to achieve similar performance to diesel fuel. However, there are several characteristics (including cetane number, oxidation stability and corrosion potential) that are of concern. These differences, especially the cetane reduction, require that adequate control of the biofuel concentration be implemented.

In addition, there are now tax incentives available in some parts of the world for the use of biodiesel. For example, in

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the USA this tax credit is presently in the form of a $0.01 per FAME-% per gallon of fuel used. Therefore, the difference between 19% or 20% FAME in diesel fuel can result in a considerable tax value. A recent investigation of commercially available biofuel blends identified that 18 out of 50 splash-blended samples were not the specified 20% FAME value (1). It can be seen that there are financial justifications for an accurate biofuel concentration determination and characterization.

This work was performed using the Spectrum™ OilExpress™ system which consists of four elements:

- The PerkinElmer® Spectrum 100 FT-IR spectrometer with high sensitivity, sampling speed, and stability.
- A sealed transmission cell with zinc selenide (ZnSe) windows with a 100-µm pathlength.
- The Molecular Spectroscopy Liquid Autosampler which provides unattended operation and rapid sample throughput of up to 50 samples per hour. The system is fitted with syringe pumps and is designed to handle samples with a wide range of vis-cosities, ensuring virtually no sam-ple carryover (<0.1%).
- The PerkinElmer infrared quantitative software suite which allows analysis by various methodologies. These include Beer's Law concentration calculations using peak height measurements and full Principal Component Regression (PCR) chemometric analysis.

The primary advantage of this system is the ability to automate the procedure from sample aspiration through report generation, including cleaning between samples. Secondly, the infrared transmission spectra carry the most information-rich data available, enabling more robust methods to be calculated.

## EN 14078 method using Beer's Law

One of the few defined methods for measuring the concentration of FAME is EN 14078 (July 2004) – "Liquid petroleum products – Determination of fatty acid methyl esters (FAME) in middle distillates – Infrared spectroscopy method" (2).

The principle of the EN 14078 method is the application of a simple quantitative model of FAME content using the 1745 cm-1 carbonyl absorbance.

When using the EN 14078 methods, samples are diluted in cyclohexane to a final analysis concentration of 0-1.14% FAME. This produces a carbonyl peak intensity range between 0.1-1.1 Abs, using a 0.5-mm cell pathlength. The peak height of the carbonyl band at or near 1745 cm-1 is measured to a baseline drawn between 1820 and 1670 cm-1. This peak height is used with a Beer's Law plot (absorbance versus concentration) to develop the calibration curve used for calculating the unknown concentrations.

While it is possible to achieve good concentration measurement, the disadvantages of this method are the need for sample dilution and the inability of the simple methodology to cope with variances in the source of the biofuel. An improved solution utilizes the more common 100-µm flow-cell, avoiding sample dilution errors. With the potential for increasing variance in feedstocks used to produce the FAME (namely:

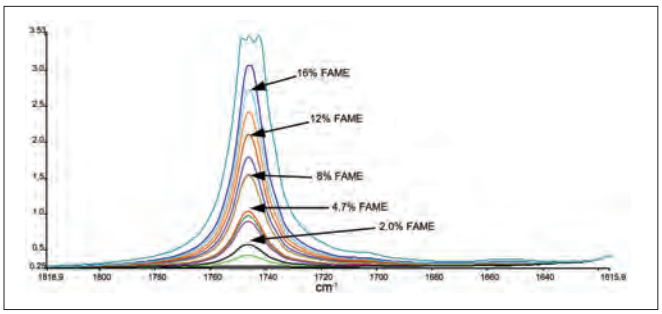


*Figure 1.* FT-IR spectra of varying FAME concentrations in diesel fuel.

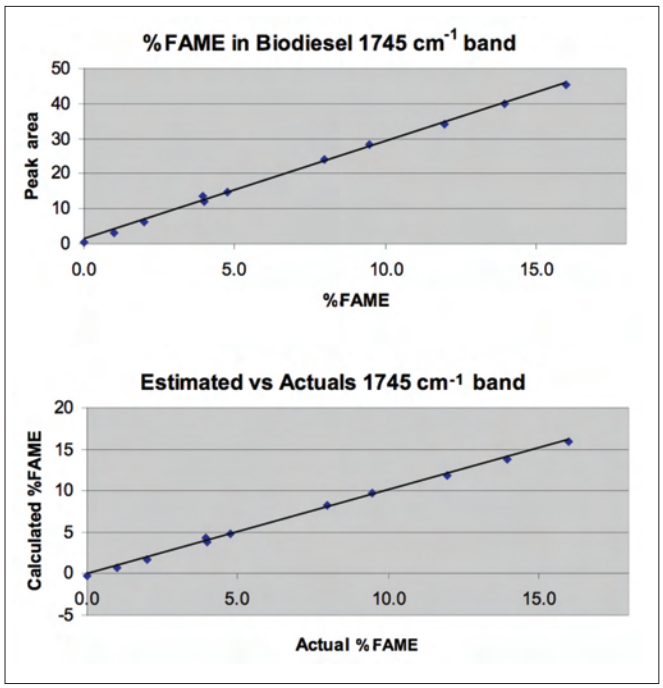


*Figure 2.* Beer's Law calibration method for 1745 cm-1 peak.

soybean, rapeseed, or yellow-grease), peak area is proposed as a preferred calculation technique.

## Peak area method

The modifications of this method that were employed in this study included:

- Cell pathlength – 0.1 mm
- Peak area calculation – range: 1820-1670 $cm^{-1}$ with baseline set at the same range.
- No dilution – samples were not diluted to allow for the determination of the usable range.

For a concentration method to be valid, the peak maximum cannot exceed the absorbance range of the spectrometer. Figures 1 and 2 demonstrate that the Beer's Law curve for this spectral region is limited to approximately 18% FAME.

In this study, we took a baseline as defined in the EN 14078 method at 1820-1670 $cm^{-1}$ and a peak area in the same range. The sample concentration range for this method was B0 to B16 (0% to 16% FAME). The method produced a linear graph with a correlation coefficient of 0.9988. Calculating the concentration of the standards by the method yielded a Pearson's correlation of 0.9990 and a standard error of prediction (SEP) of better than 0.30%. These results indicate an acceptable method for the quantitation of FAME up to B16.

Further analysis of the FT-IR spectra shows additional spectral regions attributed to the FAME chemistry; for example 1300-1130 $cm^{-1}$ (see Figure 3). The peak maximum for this spectral region does not exceed the system absorbance limit even at 49% FAME. The associated Beer's Law method uses the peak area between 1300 and 1130 $cm^{-1}$. Figure 4 shows the capability of this method for an extended sample concentration range from B0 to B50. The method produced a linear correlation with a correlation coefficient of 0.9997 and a standard error of prediction (SEP) of 0.38%. This is a capable method for the determination of a wider range of FAME concentrations.

## Principal component regression method

The peak area model is able to yield very capable calculations of the FAME concentration using short ranges of the full IR spectrum. To fully utilize all the relevant information from the whole spectrum, we moved to a chemometric analysis. In this case, we used Principal Component Regression (PCR) to provide a more robust concentration assay. Samples with varying FAME concentrations between 0 and 20% were used in the calibration of the PCR model. The model employed as much of the entire spectrum as available. The quantitative prediction utilized only one principal component (the Regression Spectrum for the method). This spectrum (Figure 5) shows that the entire spectrum was used except the top of the 1745 $cm^{-1}$ FAME carbonyl peak and the C-H peaks at the 2900, 1460, and 1370 $cm^{-1}$ region.

By using the entire spectral region, a more robust model can be generated. The statistics of this model showed a correlation coefficient of 0.9995, Pearson's correlation of 0.9997 and SEP of 0.17%. The actual against predicted results for this model as shown in Figure 6 also confirm a good prediction model.

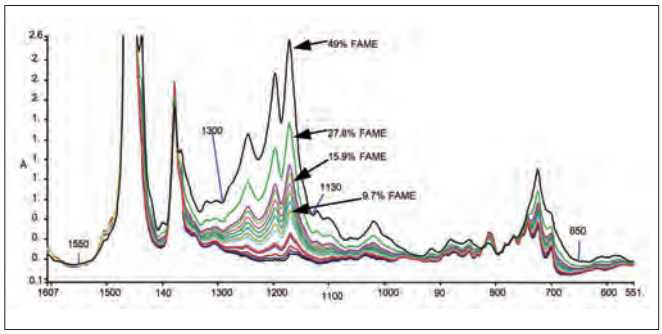


*Figure 3.* Fingerprint region of FAME/diesel samples.

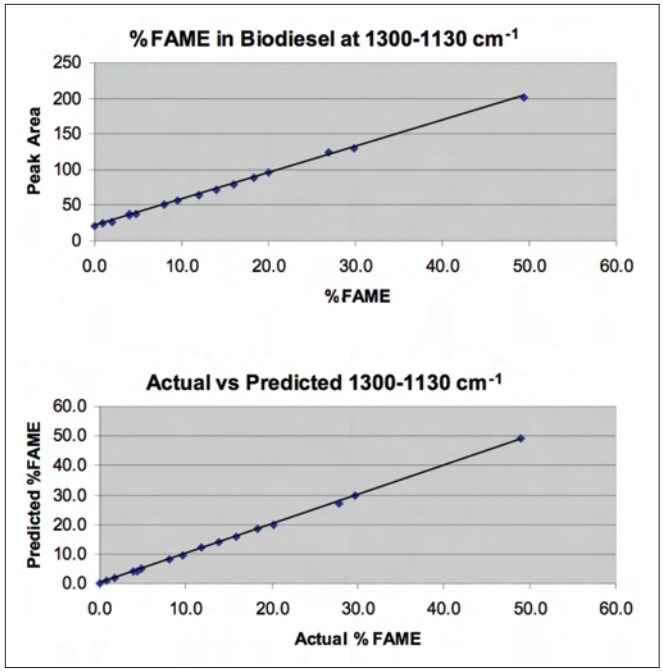


*Figure 4.* Beer's Law calibration method for 1300-1130 $cm^{-1}$.

This chemometric approach to the analyses is equal to, or better than, the Beer's Law methods. Although this modeling method for developing a calibration of the concentration of FAME in a biodiesel is more difficult to design, it is more robust over larger concentrations. Additionally, it will allow extending the calibration range with additional samples to even higher concentrations.

## Conclusion

We have shown how infrared transmission techniques can be used to address FAME concentration measurements. All the methodologies presented achieve a standard error of prediction of less than 0.4%. This compares well with the concentration measurement of FAME in a typical "splash blend" operation, where an error of 0.5% is usually acceptable. Data analysis using either Beer's Law or Principal Component Regression (PCR) is capable of meeting this requirement.

A key advantage to using the transmission cell sampling method is that it allows auto-sampling, which can ease the routine laboratory's manpower needs. The choice of either Beer's Law or chemometrics will be determined by the particular situation.The Beer's Law approach, using peak area, benefits from being a simpler approach and is recommended for situations where there are relatively few standards and low throughput of samples. The chemometrics approach has the advantage of being more robust with respect to known constituents in the blend, better handling of interferents, and reduced effect from noise contributions. Overall, PCR offers higher confidence in the quantitative prediction than is found with the Beer's Law methods.

**Note:** While the procedures provided in this Application Note may not have yet found their way into methodologies set by standard organizations or government agencies, they have been fully tested and have been demonstrated to provide quality data in numerous laboratories performing routine FAME analysis.

## References


1. NREL Technical Report TP-540-38836, *Survey of the Quality and Stability of Biodiesel and Biodiesel Blends in the United States in 2004*; R.L. McCormick, T.L. Alleman, M. Ratcliff, L. Moens, and R. Lawrence (October 2005).
2. EN 14078 *Liquid Petroleum Products – Deter-mination of Fatty Acid Methyl Esters (FAME) in Middle Distillates – Infrared Spectroscopy Method* (July 2004).
3. ASTM D7371 Standard Test Method for "Determination of Biodiesel (Fatty Acid Methyl Esters) Content in Diesel Fuel Oil Using Mid Infrared Spectroscopy".


## Acknowledgements


This work was performed for PerkinElmer by David L. Wooton, Ph.D., of Wooton-Consulting, Beaverdam, VA, USA.


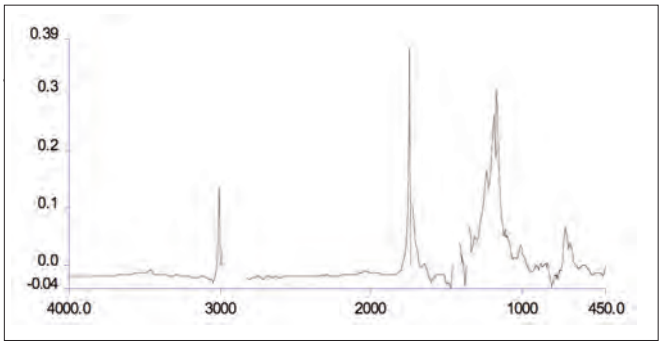


*Figure 5.* FAME PCR regression spectrum.

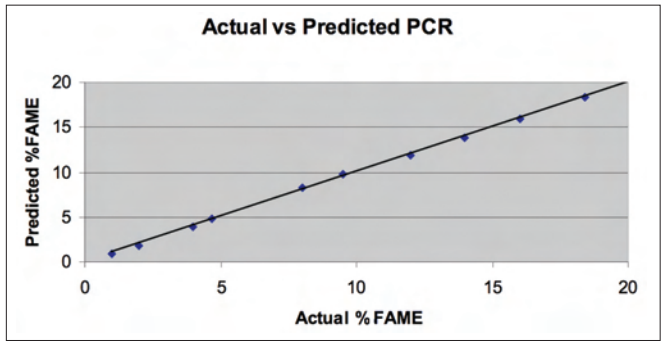


*Figure 6.* PCR calibration method.


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007845A_02

# Phosphorus, Calcium and Magnesium Analysis of Soybean Oil-Feedstock for Biodiesel Production Using the Optima Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES)

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## Introduction

Biodiesel consists of mono-alkyl esters of fatty acids derived from vegetable oil (or animal fat) and is rapidly gaining momentum in the US as an alternative fuel source for diesel engines. In Europe it is already well established. As demand for biodiesel increases in all parts of the world, new manufacturing facilities are being built at an extraordinary rate. Compared to petroleum diesel, biodiesel is environmentally friendly and is government mandated. It reduces carbon monoxide (CO), carbon dioxide ($CO_2$), sulfur dioxide ($SO_2$), hydrocarbons (HC) and other particulate matter emissions that cause respiratory damage. Biodiesel also eliminates the cloud of dense, black smoke normally associated with diesel vehicles. The exhaust fumes from an engine running biodiesel may smell like popcorn or French fries. It also has better lubricity than diesel fuel because of its higher viscosity.

Some of the benefits biodiesel has over petroleum-based diesel include:[1]

- **Requires less energy to produce:** the fossil fuel energy required to produce biodiesel from soybean oil is only 30% of the energy contained in one gallon of the fuel
- **Reduces harmful emissions:** burning biodiesel produces less $CO_2$, moreover, as soybeans grow they take-up $CO_2$. In addition, tailpipe particulate matter emissions are reduced with the use of biodiesel
- **Lower sulfur content:** most biodiesel fuels contain less than 15 ppm sulfur
- **Improved lubricity:** biodiesel is twice as viscous as petroleum-based diesel
- **Implementation is easy:** conventional diesel engines can run up to 20% biodiesel blends with no modifications

Soybean oil is fast becoming the principal feedstock to many biodiesel plants being built in the United States. However, natural variation in oil quality can affect its conversion into biodiesel. One method of determining the quality of the soybean oil is to quantify its natural occurring metallic elements. These elements principally include phosphorus, calcium and magnesium. These elements, if allowed to vary in concentration in the oil, can result in poor separation of the biodiesel esters from its co-products such as glycerin and fatty acids during production. Swiftly and accurately measuring these elements is important in verifying oil quality.

In the US, ASTM standard D6751 and in Europe, EN 14214 are used for guidance on acceptable levels of metals that may affect the performance of the final product. Table 1 summarizes the metallic content specified in the final product to ensure proper engine performance.

*Table 1.* Consensus Standards for Maximum concentrations of P, Ca, and Mg.

| Element | ASTM D6751 | EN 14214:2003 |
|---|---|---|
| P | 10 mg/kg | 10 mg/kg |
| Ca + Mg | 5.0 mg/kg | 5.0 mg/kg |
| Na + K | 5.0 mg/kg | 5.0 mg/kg |

* ASTM also specifies the method for determination of the elements. Recent expansions in the methodology specified include ICP-OES for the measurement of all five elements indicated in Table 1.(2) Sulfur must also be measured in the final product and although ICP-OES is not currently specified, it may be allowed in the future.

## Experimental

The analytical operating conditions are listed in Table 2. Approximately 1 gram of soybean oil was weighed into a 25-mL flask. The samples and standards were diluted with kerosene (Premisolve) by a factor of 10 to 20 times. The calibration curve was created using a multi-element organo-metallic standard (Conostan® S-21). Cobalt was added as an internal standard to all samples and standards.

Table 2. Instrumental Conditions

### Analytical Instrumentation

- Optima™ 2100 Dual View ICP-OES
- GemCone Nebulizer
- Quartz Cyclonic Spray Chamber
- Quartz Torch for Optima 2100
- 1.2 mm I.D. Alumina Injector for Oil Analysis

### Plasma Conditions

- Plasma Flow = 18 L/min
- Auxiliary Flow = 1.4 L/min
- Nebulizer Flow = 0.45 L/min
- RF wattage = 1500 W
- Pump Flow Rate = 1.50 mL/min
- Read Delay = 15 seconds
- Plasma Condition = Wet

### Spectral Conditions

- Background Correction = 2 point
- BGC1: P = -0.048; Ca = -0.068; Mg = -0.61; Co = -0.048
- BGC2: P = 0.045; Ca = 0.068; Mg = 0.61; Co = 0.041
- Phosphorus wavelength = 213.62
- Cobalt wavelength (Used as Internal Standard) = 228.619
- Calcium wavelength = 317.933
- Magnesium wavelength = 285.213

The plasma was viewed in the radial mode. Additional sensitivity was not required since this is a quick survey method to roughly approximate the concentration of the elements for process control. Calibration standards of 0, 0.1, 1.0 and 10 mg/kg of each element were used.

## Results

A variety of oil batches were analyzed for the elements of interest and the results shown in Table 3. The three feedstock types represent progressively refined materials progressing toward the conversion process. There are no regulations for metals in feedstock materials so these analyses are done to ensure that the manufacturing process proceeds smoothly. The instrument results take less than two minutes per sample, after a short calibration period.

The wide dynamic range of the ICP-OES provides an advantage in that it allows for the measurement of a wide range of potential sample concentrations in one run. This provides rapid turnaround, important when a process may be halted, waiting for diagnostic analytical results.

*Table 3.* Analysis of Oil Feedstock for Metallic Elements (mg/kg).

| | Plant Feed | | | Degummed Oil | | | Crude Oil | | |
|---|---|---|---|---|---|---|---|---|---|
| **Feed Stock** | **P** | **Ca** | **Mg** | **P** | **Ca** | **Mg** | **P** | **Ca** | **Mg** |
| Batch 1 | 7.07 | 0.83 | 0.79 | 30.00 | 3.63 | 3.30 | 448.5 | 57.9 | 54.7 |
| Batch 2 | 11.63 | 1.48 | 1.45 | 7.89 | 0.72 | 0.72 | 550.7 | 68.3 | 66.1 |
| Batch 3 | 8.64 | 1.10 | 1.10 | | | | | | |
| Batch 4 | 8.97 | 1.00 | 1.01 | 6.36 | 0.67 | 0.79 | 521.0 | 60.7 | 61.5 |
| Batch 5 | 6.32 | 0.86 | 0.89 | 6.87 | 0.79 | 0.82 | 625.7 | 73.3 | 67.0 |
| Batch 6 | 6.01 | 0.84 | 0.86 | 10.04 | 1.11 | 1.04 | 447.4 | 55.7 | 51.5 |
| Batch 7 | 6.33 | 0.88 | 0.83 | 12.92 | 1.39 | 1.21 | 507.4 | 63.4 | 57.0 |
| Batch 8 | 11.55 | 1.38 | 1.51 | 61.75 | 6.40 | 6.90 | 545.1 | 60.4 | 68.8 |
| Batch 9 | 11.55 | 1.38 | 1.51 | 61.75 | 6.40 | 6.90 | 545.1 | 60.4 | 68.8 |
| Batch 10 | 37.81 | 4.42 | 4.95 | | | | | | |
| Batch 11 | 16.27 | 1.84 | 2.11 | | | | | | |
| Batch 12 | 12.23 | 1.43 | 1.53 | 24.70 | 2.69 | 2.88 | 584.3 | 68.7 | 74.4 |
| Batch 13 | 7.92 | 1.02 | 1.01 | 10.00 | 1.12 | 1.04 | 583.3 | 66.8 | 61.6 |
| Batch 14 | 7.37 | 0.95 | 0.91 | 7.93 | 0.97 | 0.86 | 639.0 | 77.5 | 71.0 |
| Batch 15 | 7.96 | 0.87 | 0.87 | 9.39 | 0.95 | 0.86 | 589.1 | 63.9 | 60.1 |
| Batch 16 | 16.80 | 1.94 | 1.77 | 9.16 | 1.21 | 1.00 | 814.0 | 99.6 | 87.7 |
| Batch 17 | 16.20 | 1.77 | 1.55 | | | | | | |
| Batch 18 | 19.70 | 1.94 | 1.69 | | | | | | |
| Batch 19 | 15.10 | 1.40 | 1.20 | | | | | | |
| Batch 20 | 14.70 | 1.14 | 1.33 | 8.91 | 0.87 | 0.89 | 582.0 | 67.9 | 61.3 |
| Batch 21 | 4.78 | 0.32 | 0.42 | | | | | | |
| Batch 22 | 36.50 | 4.87 | 4.37 | 115.90 | 15.00 | 11.80 | 1286.0 | 167.7 | 151.7 |
| Batch 23 | 37.10 | 4.90 | 4.60 | 23.97 | 2.53 | 2.28 | 1218.0 | 157.2 | 149.5 |

Table 4 shows the precision for these elements measured in feedstock and refined feedstock. The samples are diluted by a factor of 10-20 so the concentrations actually measured are quite low in some cases and the precision would be expected to be poorer than at higher concentrations.

## Conclusion

The analysis of soybean oil feedstocks using the Optima ICP-OES is a fast and accurate way to measure naturally occurring elements such as phosphorus, calcium and magnesium present within the soybean oil. The presence of these elements at certain concentrations is an indicator of the quality of the feedstock for processing. The concentration in the final product is also specified by consensus groups to ensure proper engine performance. Initial measurement and on-going monitoring is an important part of product quality control protocols.

## References


1. M. Bowman, D. Hilligoss, and S. Rasmussen, and R. Thomas, Biodiesel: A Renewable and Biodegradable Fuel, Hydrocarbon Processing, February 2006, p. 103-106.

2. R. McCormick and S. Westbrook, Biodiesel and Biodiesel Blends, ASTM Standardization News, April, 2007.


*Table 4.* Precision for Biodiesel Feedstocks (n=5).

| Sample | P (mg/kg) | %RSD | Ca (mg/kg) | %RSD | Mg (mg/kg) | %RSD |
|---|---|---|---|---|---|---|
| Soy crude 2216 | 1270 | 1.0 | 175 | 0.6 | 171 | 2.0 |
| Soy refined 2218 | 9.1 | 5.2 | ND | – | 0.72 | 4.4 |
| Corn oil | 2.1 | 16 | ND | – | 0.28 | 8.4 |
| Animal fat | 234 | 2.6 | 170 | 3.6 | 20.9 | 2.6 |
| Poultry | 14.5 | 6.6 | ND | – | 0.15 | 43 |
| Check sample | 40.2 | 3.3 | 40.5 | 2.5 | 40.4 | 3.1 |

ND: not detected at an approximate detection limit of 0.4 mg/kg in the original sample.
Other elements such as sodium, potassium, and sulfur also have specifications in the final product and are not measured within the scope of this work.


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007921_01

# Sulphur and Phosphorus Analysis in Vegetable Oil and Beef Tallow for Biodiesel Production Using the Optima Inductively Coupled Plasma-Optical Emission Spectrometer

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## Introduction

Renewable biological wastes, such as animal fats and vegetable oils, are gaining interest in the energy industry sector as potential alternative fuel sources for diesel engines. Feasibility of use depends on many factors including:

1. Physical Properties

The specific energy densities (energy content per fuel mass) of vegetable oils and animal fats (~40MJ/kg) are approximately 80% of diesel (~48MJ/kg). Minimum temperatures at which biodiesels are useable increases at higher concentrations (10-20%)[1]; these temperatures and viscosities also increase with greater degrees of saturation, going from vegetable to animal sources. Hence, biodiesel is often used and blended with diesel. Besides reducing fuels costs, when added in small quantities (<3%) to petroleum-based diesel, biodiesels provide better lubricity, improve operation of diesel equipment and extend component life. At these low quantities, cold temperature properties (i.e., cloud point, cold filter plugging and pour point) are not affected. Biodiesels also exhibit low reactivity with other materials such as reduced copper corrosion[2].

2. Environmental Impact and Safety

Unlike virgin vegetable oils, rendered products such as edible and inedible tallow, greases and lard, are recycled waste products and they will not increase demand of raw materials. In general, biodiesels emit less HC, COx, and SOx, than petroleum-based diesel for the same amount of fuel used. Biodiesels have high boiling points and flash points, very low vapor pressures, and a lesser tendency to smoke. This indicates a high level of safety in handling[2].

3. Costs[3]

Market price of raw materials, transportation costs, quality, supply and reliability are some of the obvious costs of procurement. Compared to petroleum-based diesel, raw material and production costs per gallon of animal-based and grease-based biodiesels are similar or lower; brown grease is about half the cost. Plant material (corn, soy, canola) costs, however, are higher; corn is about twice the cost. Unlike refined oils, animal fats require prior degumming, bleaching, thermal deacidification and esterification. Biodiesel yield, storage, oxidation stability, disposal cost, labor and infrastructure are some of the other costs of operating a biodiesel plant.

4. Testing

Given the diversity of sources of biofuels, testing of raw materials is an important first step in assuring consistent quality of the final product. Fuel sulphur is converted to sulphur oxides and sulphuric acid. Although raw materials may not contain large quantities of sulphur, some processing methods use sulphur catalysts to utilize fatty acid feedstocks[2].

Phosphorus damages catalytic converters used in emission control systems. Since catalytic converters are becoming more common in diesel powered equipment, maintaining low or no S and P levels will be of increasing importance.

European and U.S. Biodiesel Specifications for some elements are listed in Table 1.

*Table 1.* European and U.S. Biodiesel Specifications (% mass units).

| | ASTM D-6751 | pr EN 14214 | E DIN 51606 |
|---|---|---|---|
| Phosphorus | 0.001 | 0.001 | 0.001 |
| Sulphur | 0.05 | 0.01 | 0.01 |
| Ca + Mg | — | 0.0005 | 0.0005 |
| Na + K | — | 0.0005 | 0.0005 |

Procedures for determining S and P in beef tallow and canola oil are described below.

## Experimental

1. Polypropylene centrifuge Tubes, 50 mL, Sarstedt or equivalent
2. Vortex – Fisher Scientific Ltd. Model-G560 or equivalent
3. Transfer pipette, disposable or otherwise.
4. Mettler-Toledo, Model PG 1003-S weighing balance or equivalent

### Reagents

The calibration standard for P, S and Mn was made using Conostan® standards. (Conostan Division, Conoco, Inc. P.O. Box 1267, Ponca City, OK 74601). A 10 ppm calibration standard was used.

The solvent used in all cases was a mixture of 90% PremiSolv kerosene (Conostan) and 10% hexane (A.C.S. Reagent, Anachemia, 45126-540, UN-1208) for beef tallow. Hexane was not necessary for canola oil.

### Sample preparation

Transfer 2 g of tallow using a spatula to a 50 mL centrifuge test tube. Using a Pasteur pipette, add 2 g of hexane to the tube. Add 0.2 g 100 ppm Mn and add PremiSolv to 20 g total. Vortex until the tallow is completely dissolved, approximately 1 to 2 minutes. Let solution stand until all bubbles are dissipated and the solution clarifies, about 3 minutes. All samples, standards and controls for tallow were diluted with kerosene/hexane (90/10) prior to measurement.

Preparation of canola oil is a dilution of 1 g canola oil and 9 g PremiSolv, followed by vortex. All samples, standards and controls for canola oil were diluted with kerosene prior to measurement.

A final concentration of 1 ppm Mn was used as an internal standard to correct for physical interferences affecting P and S. This was added to the kerosene/hexane solvent for tallow, and kerosene only for canola oil. A higher concentration may be required for other systems; a minimum of 100,000 counts per second is required. The Mn solution is stable for three days.

### Instrument conditions

All ICP-OES data were collected using the PerkinElmer® Optima™ 2100 DV ICP-OES and an AS 93plus autosampler.

1. Low flow GemCone™ Nebulizer (N069-0671)
2. Standard single slot torch (N077 0338)

Modifications to the standard system are as follows:

1. 2 mm ID ceramic injector was replaced with a 1.2 mm ID straight bore injector (N077 6093)
2. 2 mm ID injector adapter holder was replaced with a 1.2 mm ID injector adapter holder (N077 6091)
3. Standard cyclonic spray chamber was replaced with the 4 mm baffled cyclonic spray chamber (N077 6090) which requires a black holder (N077 0614) if currently using a Scott spray chamber

Solvent flex peristaltic pump tubing is used: black/black for sample and red/red for the drain. The flush was not used. Read times were 2 seconds minimum and 5 seconds maximum. Three replicate measurements were used.

*Table 2.* Instrument Conditions.

| | |
|---|---|
| RF Power | 1500 W |
| Plasma Gas | 15 L/min |
| Auxiliary Gas | 2.0 L/min |
| Nebulizer Gas | 0.3 L/min |
| Pump Speed | 0.6 mL/min |
| Purge | High |
| Torch Cassette Position | -3.5 |
| Read Delay | 180 seconds |

### Spectral conditions

Emissions at low ultraviolet wavelengths (<185 nm) from the ICP are absorbed by oxygen in ambient air. The air is purged out of the optical path by means of a high purge of either argon or nitrogen which does not affect transmission of the low UV. Displacement of air by nitrogen to steady state conditions requires approximately 1.5 hours from system ready[4]. Determination of trace concentrations of S must be done axially in order to

maximize the sensitivity. Absorption of low UV by the thin shear gas (air) that removes the plasma plume is negligible compared to the entire light path from ICP to detector. The shear gas also serves to considerably reduce material deposition on the entrance window.

A five point peak area is used for quantitation. Spectral interference correction was not required for this analysis.

*Table 3.* Operating Parameters.

| Element | Wavelength (nm) | Background (nm) |
|---|---|---|
| P | 213.617 | -0.031 |
| S | 180.669 | -0.048 |
| Mn | 257.610 | -0.054 |

### Physical interferences

Fats and oils have widely varying densities, viscosities and surface tension which result in different nebulization efficiencies. For accurate measurement, these differences must be corrected. Matrix matching of calibration standards and samples can be achieved fairly successfully by diluting samples with a solvent such as kerosene, or mixtures of kerosene and xylene, or in this case, kerosene and hexane. A commonly used dilution factor of 1 part sample and 9 parts solvent results in 10% of the testing material not exactly matched to the calibrant. This 10% can be accounted for to some extent with internal standardization. Prior studies using a reference material for edible oils indicated that manganese is a better internal standard than the commonly used Co or Y. The canola was used for COPA (Canadian Oilseed Processors Association) certification. Internal standards provide a monitor on changes of conditions in the sample introduction system. Samples that cause a greater than 25% difference from the calibrant are additionally diluted to reduce the matrix effect.

## Results

There are no certified reference materials for P and S in tallow. Reference results for S in five separate samplings of a tallow sample were provided by POS Pilot Plant Corporation (118 Veterinary Road, Saskatoon, Sask. Canada, S7N 2R4) using an Optima 4300 DV ICP.

Reference results for P in one tallow sample was provided by Dr. A. Verwey Chemical Laboratories (Coolhaven 32, 3024 AC Rotterdam, The Netherlands, P.O. Box 6003,3002 AA Rotterdam, http://www.drverwey.nl) using ICP-OES.

Results compare well as shown in Table 4.

A crude degummed canola oil reference material (ID 05-183, Cargill Ltd., P.O. Box 190 Cheviot Rd., Clavet, Sask., S0K 0Y0, Canada) was utilized for QC purposes. Three aliquots of beef tallow were sampled and prepared separately. An additional sample was spiked with a final concentration of 9 ppm P and S. These results are shown in Table 5 and represent concentrations in the raw material. The actual concentrations measured are 10 times lower.

*Table 4.* Reference Material Results, ppm

| | Sulphur 3P | Sulphur 8P | Sulphur 10P | Sulphur 8S | Sulphur 10S | Phosphorus |
|---|---|---|---|---|---|---|
| IMS | 13.5 | 14 | 15 | 14 | 14 | 191 |
| POS | 13.9 | 14.1 | 14.3 | 13.5 | 14.2 | — |
| Verwey | — | — | — | — | — | 199 |

*Table 5.* Sample Results.

| Sample | S (% mass) | P (% mass) | Mn (% recovery) |
|---|---|---|---|
| QC1 | 0.00144 | | 120 |
| Expected QC | 0.0014 | | |
| QC2 | | 0.0198 | 114 |
| Expected QC | | 0.0200 | |
| A1 | 0.00165 | 0.00424 | 106 |
| A1 duplicate | 0.00148 | 0.00423 | 105 |
| A2 | 0.00135 | 0.00439 | 101 |
| A2 spiked with 9 ppm P and S | 0.00978 | 0.0125 | 100 |
| Spike Recovery (%) | 94 | 93 | |

## Conclusion

The European Union leads the way to environmental protection and implementation of biodiesel production. At 100 ppm maximum tolerance for S and P, i.e., 10 ppm in the plasma after dilution, an axial view ensures the ICP system would be able to reliably meet anticipated lower regulation levels now and in the future. The Optima ICP-OES provides reliable and accurate analysis of sulphur and phosphorus in canola oil and beef tallow. In addition, the Optima can be easily set up to also measure Ca, Mg, Na, and K. Sodium and potassium must be measured radially to avoid any issues with ionization interferences amongst the alkali metals.

## References


1. Biodiesel: A Renewable and Biodegradable Fuel, M. BOWMAN, D. HILLIGOSS and S. RASMUSSEN, PerkinElmer Life and Analytical Sciences, Shelton, Connecticut, and R. THOMAS, Scientific Solutions, Washington, DC.
2. Production of Biodiesels from Multiple Feedstocks and Properties of Biodiesels and Biodiesel/Diesel Blends. Report 1. J.A.Kinast, National Renewable Energy Laboratory, US Dept of Energy Laboratory, Colorado, 2003.
3. Economics of a Biodiesel Plant, Martin Kleber, mg engineering Lurgi PSI, AOCS, May 10, 2004.
4. Light and It's Measurement: Part 2 Sensitivity and Stability, Pamela Wee, PerkinElmer LAS Canada ICPOES /ICPMS Users Group Meetings held in Quebec City (2005), Toronto (2005), Edmonton (2006), and Vancouver (2006).



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BIODIESEL MAGAZINE

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LAB

# Weighing Oxidation Stability Tests for Biodiesel

By Peng Ye

Biodiesel is an alterative fuel derived primarily from vegetable oil or animal fat. It consists of mono-alkyl esters of saturated or unsaturated long-chain fatty acid, depending on the feedstock. Unsaturated fatty acid alkyl ester-like linoleic and linolenic acid esters are more susceptible to oxidation than saturated fatty acid ester. As a result, biodiesel can become oxidized by the oxygen in the air during storage. The oxidation rate can be influenced by many factors such as temperature and chemical composition. Oxidative degradation is harmful and can deteriorate many physical properties of the biodiesel including viscosity, and acid and peroxide values. Antioxidants such as alpha-tocopherol or tert-butylhydroquinone are often added to increase the oxidation stability of biodiesel.

Differential scanning calorimetry (DSC) is a well-established technique to characterize the physical properties of oils including petroleum and vegetable oils. Since the oxidation reaction is exothermic, DSC can be used to study the oxidation stability of biodiesel. The method is called oxidation induction time (OIT), and it has been used to evaluate the oxidative stability of petroleum oil. This test is conducted under accelerated conditions (e.g., high temperature or high pressure) in order to shorten the experimental time to minutes instead of hours or days, which is required under normal storage conditions. The pressurized differential scanning calorimetry is specially designed to conduct the DSC measurement under elevated pressure. It has been used to study polymer phase transition and polymerization reaction and the oxidation stability of lubricating oil.

## Oxidation Testing

For the following experiment, OIT testing of four different biodiesel samples using pressure differential scanning calorimetry was performed to determine compliance with ASTM D 6186 standard test methods. Elevated pressure was employed to accelerate the oxidation reaction and suppress the evaporation of the biodiesel at high temperature. Meanwhile, the oxidation onset temperature (OT) was also

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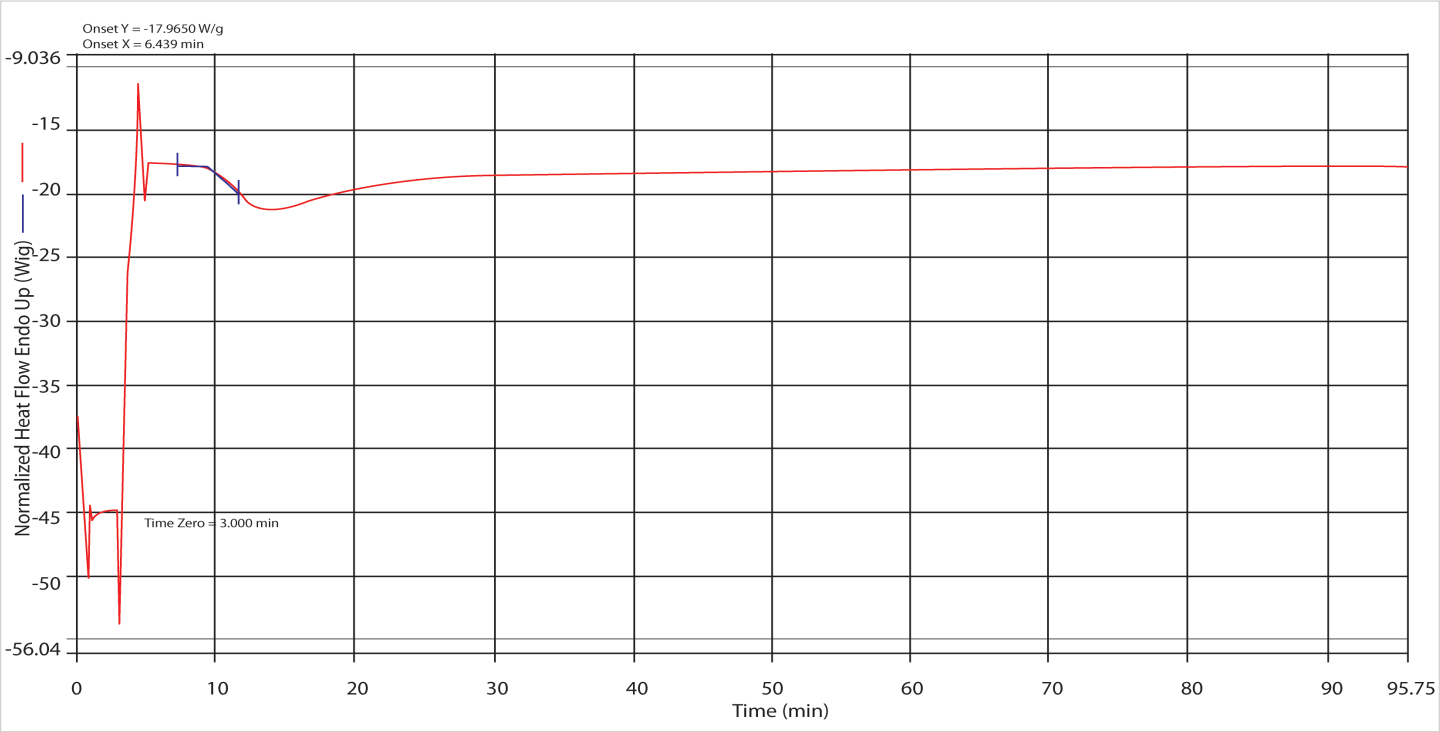


Figure 1. OIT of sample 3 rice biodiesel at 130 degrees Celsius

SOURCE: PERKINELMER

determined using pressurized differential scanning calorimetry.

The instrument used was a PerkinElmer Diamond DSC with a high-pressure cell. The instrument was calibrated using indium under the experimental condition.

The biodiesel tested included samples 1 and 2 from animal sources, sample 3 from rice biodiesel and sample 4 from soy-based biodiesel.

The conditions included a minimum purity of 99.5 percent oxygen and an operation pressure of 500 plus or minus 25 pounds per square inch (psi). The purge rate was 100 plus or minus 10 milliliters per minute (ml/min).

The test temperatures were 210, 180, 155 or 130 degrees Celsius.

The following describes the test method. For OIT test ASTM D 6186, 3 plus or minus 0.2 milligrams of biodiesel was weighted into a new aluminum sample pan without the cover. Beginning at ambient temperature, the test temperature was ramped to 100 degrees Celsius per minute and held for two minutes. The oxygen valve was opened to slowly pressurize the cell to 500 plus or minus 25 psi within two minutes. As soon as the pressure equilibrated, the cell purge rate was checked, adjusted and maintained at 100 plus or minus 10 ml/min. The OIT time was measured from the time the oxygen valve was opened.

For the OT test, 3 to 3.5 milligrams of biodiesel was weighted into a new aluminum sample pan without the cover. The pressure was adjusted to 500 plus or minus 25 psi and the purge rate to 100 plus or minus 10 ml/min. Beginning at ambient temperature, the pressure was held for two minutes then ramped to 220 degrees Celsius at 10 degrees per minute. The onset temperature was calculated and recorded.

## Results

Sample 3, rice biodiesel, is used as an example to illustrate the results. The OIT test started at 210 degrees Celsius, following the ASTM D 6186 method. The data indicated that the oxidation reaction happened quickly after the oxygen valve was opened. According to the standard, if the OIT is less than 10 minutes, then lower the temperature to the next level and repeat the experiment. Consequently, the same test was



BIODIESEL MAGAZINE

| Sample | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Oxidation onset temperature (degrees Celsius) | 155 | 167 | 155 | 134 |

Table 1. OT summary
SOURCE: PERKINELMER

conducted at 180, 155 and 130 degrees Celsius.

The OIT can not be readily measured until 130 degrees Celsius with an OIT of 6.4 minutes, still a relatively short period of time (Figure 1). In this figure, the first minute is the temperature ramp-up from room temperature to test temperature. The next two minutes are isothermal at the test temperature. The oxygen valve was opened at three minutes. From three to five minutes, the cell was gradually pressurized to 500 psi and the purge rate was adjusted to 100 ml/min. Note, the software used takes into consideration the zero time, which is the moment the oxygen valve was opened when performing the OIT calculation.

Further reducing the experimental temperature may result in a longer OIT. When the temperature is set to 110 degrees Celsius the OIT is calculated to be 34.6 minutes. The repeatability of this test was checked by running the same method on sample 3 five times. The results are 6.439, 6.442, 6.326, 6.440 and 6.372 minutes respectively with an average of 6.404 minutes and a standard deviation of 0.053 minutes. The DSC with the high-pressure cell gives highly repeatable results under identical experimental conditions.

Although, generally speaking, it is not possible to correlate the oxidation induction time directly to the oxidation onset temperature, the OT measurement gives another way to look at the oxidation stability of the material. In order to determine the oxidation onset temperature, the sample is scanned from low temperature to high temperature instead of being held isothermally, and the OT is determined as the onset temperature of exothermic reaction. For example, sample 3 was heated from room temperature to 220 degrees Celsius under the same experimental conditions (pressure 500 psi, oxygen 100 ml/min). The onset temperature was found to be approximately 155 degrees Celsius. The OT results for all four samples are summarized in Table 1. Clearly, the OT sequence is sample 2 is greater than sample 1 equals sample 3 which is greater than sample 4.

The OIT results are summarized in Table 2. The measurement temperature was 130 degrees Celsius for samples 1, 3 and 4 and 155 degrees Celsius for sample 2. For sample 2 at 130 degrees Celsius, no obvious OIT was detected within the measurement time of 120 minutes. Again, samples 1 and 3 performed similarly. Note, for samples 2 and 4 the oxidation happened so quickly after the oxygen valve opened that their OIT can not be determined accurately.

## Conclusion

The OIT test following ASTM standard D 6186 or OT test can be used to study the oxidation stability of biodiesel. The use of pressure DSC can significantly reduce the experimental time under accelerating conditions. Therefore, pressurized differential scanning calorimetry may be a useful tool to screen different antioxidants or different antioxidant concentrations for biodiesel fuel. ■

Peng Ye is an applications scientist with PerkinElmer Life and Analytical Sciences. Reach him at peng.ye@perkinelmer.com or (203) 402-1708.

| Sample | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Oxidation induction time (minutes) | 6.5 at 130 degrees Celsius | Less than 2 at 155 degrees Celsius | 6.4 at 130 degrees Celsius | Less than 2 at 130 degrees Celsius |

Table 2. OIT summary
SOURCE: PERKINELMER



BIODIESEL MAGAZINE

# Bioethanol Applications

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# HPLC Analysis for the Monitoring of Fermentation Broth During Ethanol Production as a Biofuel

**Authors**
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Wilhad M. Reuter
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## Introduction

Increased ethanol production as a biofuel is leading to a paradigm shift around the world. Renewable biological resources that can be converted to biofuels are rapidly gaining interest in the energy industry as potential alternative fuel sources. This is not just a U.S. phenomenon – it is accelerating globally. In particular, resources such as corn, sugar beets, sugar cane, grains, sorghum, molasses and others (all renewable energy sources) are being converted into ethanol at an ever increasing scale.

The production of ethanol utilizes a fermentation process, in which yeast and enzymes convert the fermentable carbohydrates (dextrin, maltotriose, maltose, glucose) into ethanol. The resulting fermentation broth is a complex mixture, consisting of living yeast cells, nutrients, bacteria, cell debris and other products/byproducts of the fermentation process. This broth needs to be monitored to optimize the quantity and quality of ethanol being produced. During the fermentation, it is known that the ethanol concentration is inversely proportional to the carbohydrate concentration. Therefore, the monitoring of carbohydrate levels serves as a key indicator in determining when to stop the process. In addition, other unwanted byproducts, such as lactic acid, acetic acid, carbonic acid and glycerol are also produced. To maintain productivity, these byproducts must also be monitored. During fermentation, as the composition of the broth changes, so does the chemistry. Therefore, adjustments to the fermentation broth are often required to ensure optimal ethanol yields.

This HPLC application has been designed so that, during the fermentation process, three key parameters, including eight components, can be easily monitored and quantitatively analyzed:

1) The amount of ethanol being produced
2) The amount of fermentable sugars (dextrin, maltotriose, maltose and glucose) in the fermentation broth
3) The concentration of unwanted byproducts (lactic acid, acetic acid and glycerol) produced during the fermentation process

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precisely.

## Experimental conditions

The application was performed on a PerkinElmer® Series 200 HPLC System, consisting of an Isocratic Pump, Vacuum Degasser, Autosampler, Column Oven and Refractive Index Detector. TotalChrom® Chromatography Data Systems (CDS), version 6.3.1, was used as the control/data-acquisition software. The column used was a BIO-RAD Aminex® Fermentation Monitor column (150 x 7.8 mm, 5 µm).

The analytical conditions, shown below, were optimized to produce the shortest analysis time, while maintaining sufficient resolution between components for proper identification and quantification. Using these conditions, all components can be quantitatively analyzed in less than 10 minutes.

*Table 1.* Conditions.

| | |
|---|---|
| Mobile Phase: | 0.001 M $H_2SO_4$ |
| Flow: | 0.8 mL/min |
| Temperature: | 60 °C |
| Detector: | Refractive index @ 40 °C |
| Injection Volume: | 10 µL |

## Results

An example of an actual 24-hour fermentation-broth sample that was taken during ethanol production is shown in Figure 1. From the chromatogram, it can be seen that the ethanol is well separated from all the other individually separated sugars and byproducts found in this particular sample.

## Conclusion

In conclusion, as part of the fermentation process in the production of ethanol as a biofuel, a simple ten-minute HPLC method was developed to routinely monitor ethanol, carbohydrates and byproducts. During the process, this analysis is important to help ensure that the broth chemistry is optimized to produce the maximum yield of ethanol.

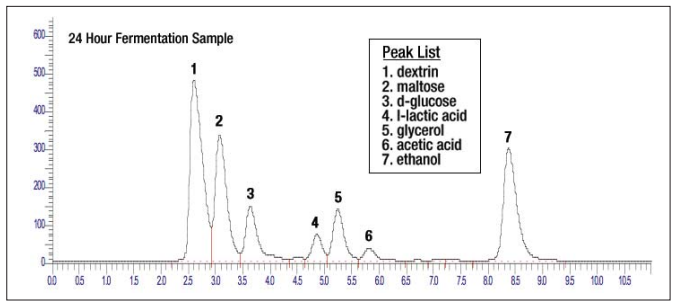


*Figure 1.* Actual 24-hour fermentation sample from ethanol production monitoring.

## Reference

U.S. Department of Agriculture – *www.usda.gov*

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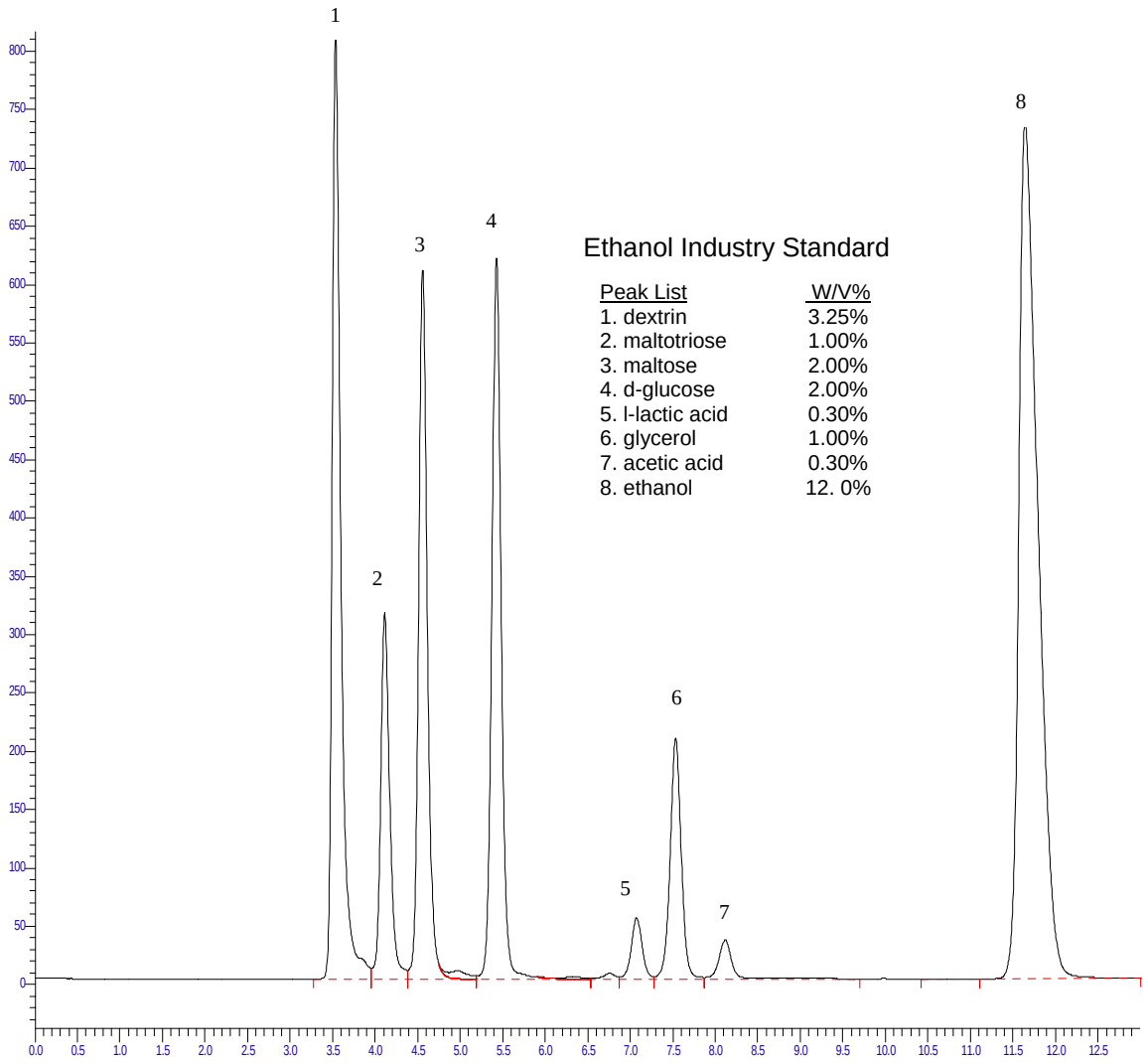
# Fermentation Monitoring




| Conditions | |
|---|---|
| Mobile phase: | 0.005M $H_2SO_4$ |
| Flow: | 1.2 mL/min |
| Temperature: | 80°C |
| Detector: | Refractive index @ 40°C |
| Injection volume: | 15 µL |

| Column | |
|---|---|
| Supelco column: | Supelcogel C-610H |
| Dimensions: | 300 x 7.8 mm |
| Part number | 59320-U |

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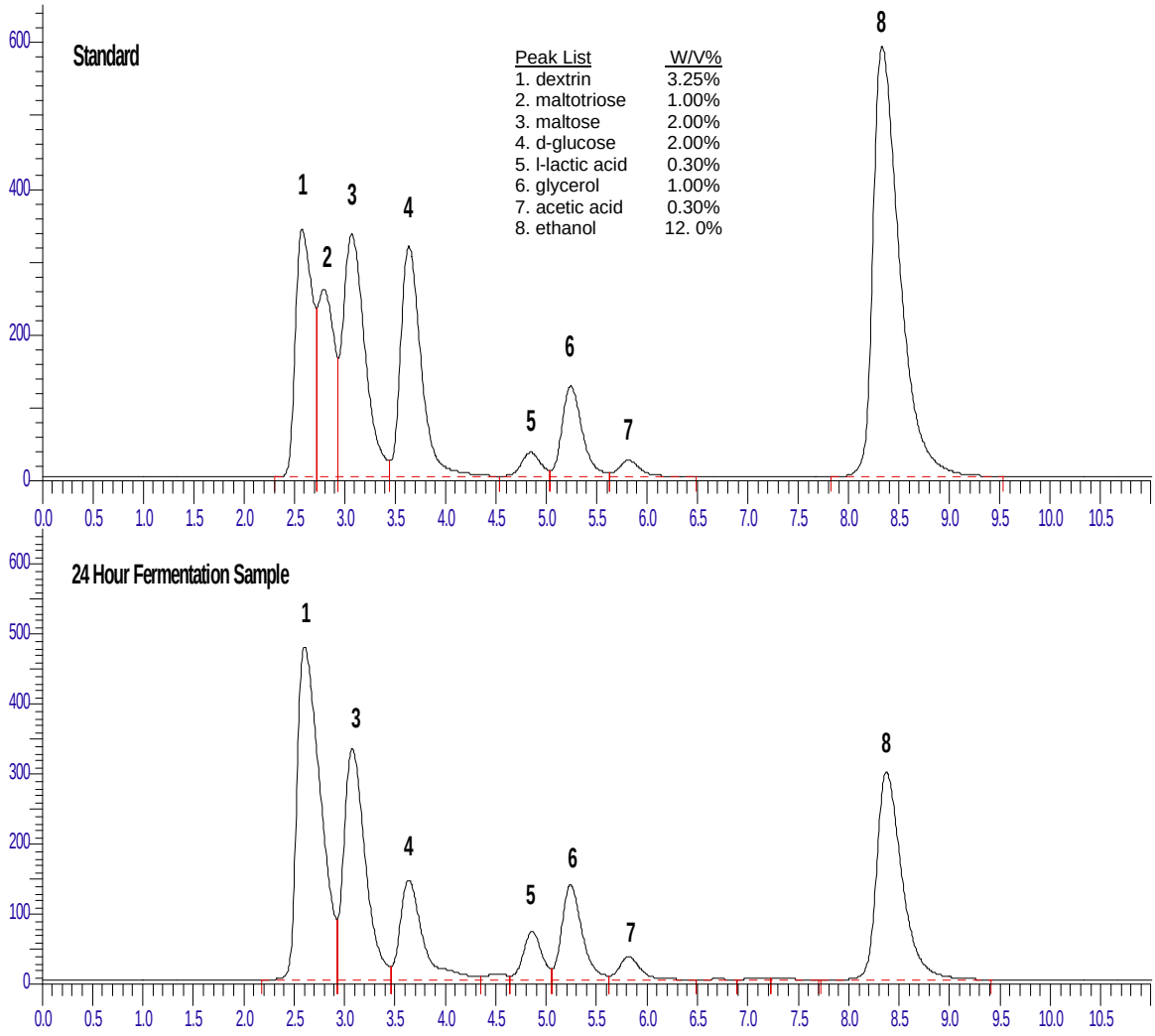
# Fermentation Monitoring




| Conditions | |
|---|---|
| Mobile phase: | 0.001M $H_2SO_4$ |
| Flow: | 0.8 mL/min |
| Temperature: | 60°C |
| Detector: | Refractive index @ 40°C |
| Injection volume: | 10 µL |

| Column | |
|---|---|
| BIO-RAD column: | Aminex® Fermentation Monitor |
| Dimensions: | 150 x 7.8 mm |
| Part number | 1250115 |

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# LSC
# Application Note 43

May 2007

## Determination of the $^{14}C$ content in fuels containing bioethanol and other biogenic materials with liquid scintillation counting

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### Introduction

The limited resources of fossil energies such as coal, oil and gas are generally known. These resources will only be able to deliver the necessary amount of energy within the next few decades. Especially the heavily increasing use of fossil sources in Asia and other rapidly growing markets and the already high level of burning fossil sources in the western industrial nations will result in a shortage of these essential materials. We already made the experience that the increasing use of fossil sources followed a strong increase in prices for consumers. These lead to a search for alternative energy sources during recent years.

Another challenge is the attempt to reduce the emission of $CO_2$ to avoid a further increase of the temperature in the atmosphere. Carbon dioxide has been generally accepted as one potential source of the green house effect although we still need further information to fully understand the complex mechanisms that result in the global temperature increase. To stop this increase in temperature many countries agreed in the Kyoto protocol in a $CO_2$ reduction of their emissions over the next years.

One possibility to make additional source of energy available for a longer time and to reduce the emission of fossil carbon dioxide is the use of renewable (biogenic) sources.[1)] The production of energy from sugar cane, rape, corn and other biogenic materials is far away from the research phase and a number of biogenic products will already be added to fossil fuels.

The regulation 2003/30/EC from the EU determines the minimum amount of biogenic materials in fuel. Until 2005 all fuels should contain at least 2% of biofuels and this should increase until 2010 to 5.75%. The current European norm for Otto fuels is EN DIN 228 which already allows the use of up to 5% of bioethanol. For diesel the corresponding norm is EN DIN 590, for biodiesel the current norm is EN DIN 14214, which has been introduced in Germany on the 30$^{th}$ of October 2004.

This application note will show in more detail the possibilities to determine the amount of biogenic materials in mixtures of fossil and biogenic materials with the help of the liquid scintillation counting (LSC) method. A very accurate method for the quantification of the biogenic amount in fuels is very important for producers as well as for custom departments in the different countries.

### What is the basic principle of the quantification of biogenic material?

Living organisms take up carbon with their food or via breathing or photo synthesis. During these processes different carbon isotopes such as the stable nuclides $^{12}C$ and $^{13}C$ as well as the radioactive nuclide $^{14}C$ will be incorporated in organic material in the exact same proportions in which they occur in nature.

We can assume that the amount of the radioactive nuclide $^{14}C$ in the atmosphere is constant during the growth period of plants because the production of $^{14}C$ via neutron capture of $^{14}N$ is in equilibrium with the radioactive decay of $^{14}C$. This is true as long as the plant growth is fast compared to the $^{14}C$ activity fluctuations in the atmosphere. Most plants for biofuel production will be harvested within one year and are therefore not influence by long term $^{14}C$ activity changes. Trees which might grow over decades can show higher amounts of $^{14}C$ in the tree rings of the 60th due to the atom bomb testing. As long as a living organism takes up carbon we have an equilibrium activity of $^{14}C$ because decay and uptake of $^{14}C$ is in equilibrium.

As soon as an organism dies or you harvest a plant the uptake of carbon stops. From this point on the original amount of $^{14}C$ decays and the current activity of this material is only dependent on the half life of this isotope. Because $^{14}C$ has a half life of 5730 years half of the original activity will be decayed after 5730 years. Currently the most sensitive detection methods for $^{14}C$ can detect 14C even in samples which are already 10 half life's old, which is approximately an age of 60 000 years. In older samples $^{14}C$ can not be detected anymore.

Because in fossil materials or in products prepared from fossil materials such as all mineral oil products the $^{14}C$ contents could decay over million of years no $^{14}C$ can be detected anymore. On the other hand in biogenic material all $^{14}C$ is still present. This difference in 14C activity can be used to determine the amount of biogenic material in fuel. Figure 1 demonstrates the $^{14}C$ circle in nature.

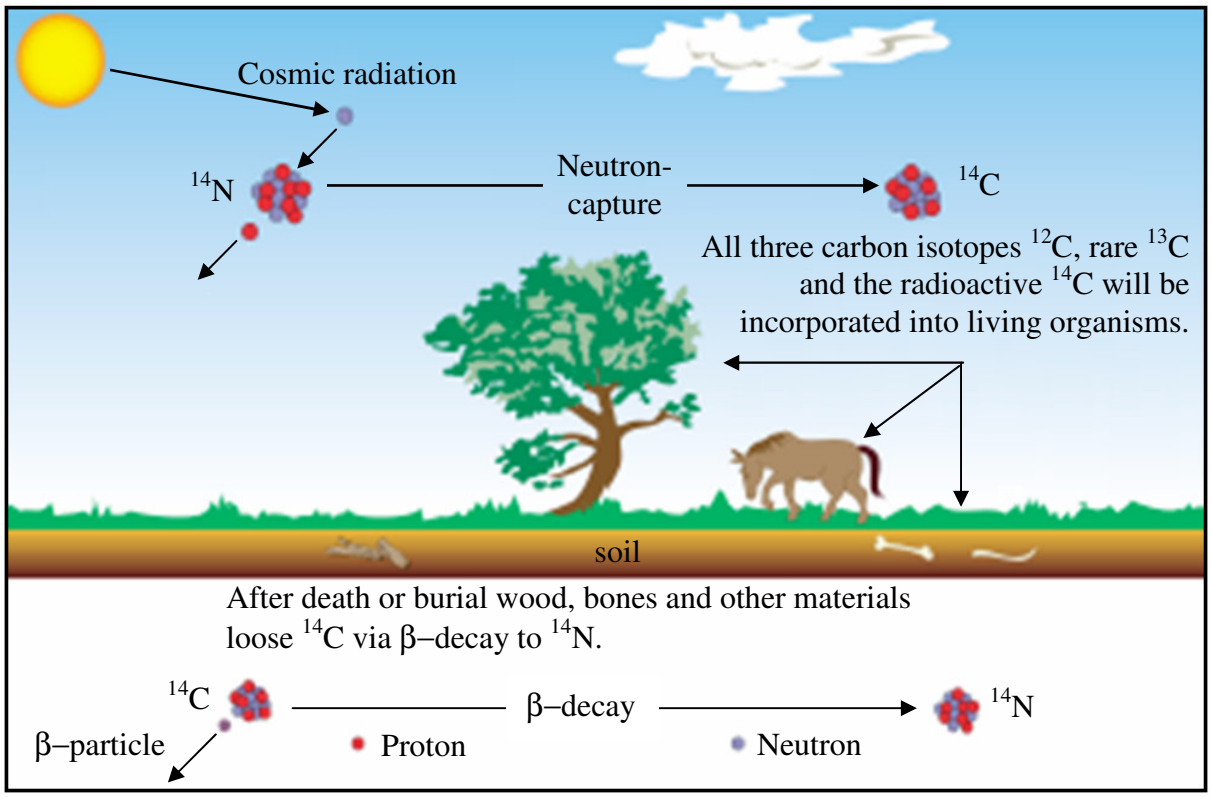



**Figure 1: Production and incorporation of $^{14}C$ in organic matter**

An assumption we have always to make is that samples must only contain mixtures of fossil and biogenic materials. A contamination with older $^{14}C$ samples (for example from trees) should be avoided.

To allow inter laboratory comparisons and comparisons between samples from different time periods result will be published in many cases as % m or % mc (% modern or % modern carbon).

In this case the amount of $^{14}C$ atoms will be determined relative to the year 1950.[2)]
As a reference material a sample from 1950 will be used which showed an activity of 13.56 ± 0.70 DPM/g carbon.[3,4)]
If you do not determine the amount of $^{14}C$ in your samples relative to 1950 but as a percentage of the current $^{14}C$ activity you have to know that this includes a higher activity due to the atom bomb tests. In this case you have to use an activity of 14.62 DPM/g carbon.[5)]

**Methods to determine the amount of biogenic material**

In general two methods can be used which are sensitive enough to detect low activities of $^{14}C$. Both methods will be described in detail in ASTM method D 6866-06 and can be downloaded from www.astm.org.
One method describes the use of AMS (Accelerator Mass Spectrometry) or IRMS (Isotope Ratio Mass Spectrometry).
The other method uses liquid scintillation counting. We will concentrate on the following pages on this latter method. The LSC technology allows using three different procedures for the determination of 14C in fuel:

Method A: Measurement of $CO_2$ in a LSC.
Method B: Use of a mass spectrometer.
Method C: Measurement of benzene in a LSC.
Method D: Direct measurement of the organic sample in a LSC.
Method A and C are especially interesting for liquid scintillation counting in case the sample has been prepared by sample combustion or sample burning. In case of method C the resulting carbon dioxide will be converted via several steps into benzene. As a consequence of this reaction you can get much higher carbon content in your sample resulting in much higher sensitivity. Also benzene is already a very good solvent for LSC measurements and you only add scintillators to your sample allowing you to make full use of your vial volume for your sample. However, it should be mentioned here, that the use of the benzene synthesis method needs a high degree of experience with this method and usually it is not possible to introduce this method in a laboratory right away.
The direct measurement of an organic sample in the LSC is always advantageous if a sample such as biofuels can be dissolved in the scintillation cocktail in any possible ratio. The organic sample should also show no or only little colour and the amount of biogenic material should be in the range of at least 1% (in case 50% carbon in the sample). Carbon content of below 1% would result in extremely long counting times or large standard deviations. Advantages and disadvantages of the four different methods will be explained in Table 1.

| Method | Advantage | Disadvantage |
|---|---|---|
| Method A: $CO_2$ in LSC | Less sample preparation and low costs compared to method C, good instrument availability. | Low sample activity due to limited sample capacity of CarboSorb E. Not sensitive for lowest $^{14}C$ activities. |
| Method B: AMS | High sensitivity, very precise. | High costs, therefore mainly for samples with carbon content below 10%. |
| Method C: Benzene in LSC | High sensitivity, very precise, good instrument availability. | More time consuming sample preparation, low capacity, benzene is cancerogenic material. |
| Method D: Direct measurement in LSC | Minimum, very fast sample preparation, good sensitivity, low costs per measurement, good instrument availability. | Keine offiziell standardisierte Methode nach ASTM 6866-06 verfügbar. |

**Table 1: Comparison of Advantages and Disadvantages between methods A – D.**

So far no standardized methods are available for method D (no part of method ASTM D 6866-06) although the use of this method for biofuels is obvious. In the meantime some investigations clearly show that method D is a suitable method for the quantification of biogenic material.[6, 7, 8)]

For further information about method A and C please read the literature.[9, 10)] Table 2 illustrates so approximated costs and necessary time for the different methods.

| Method | Sample preparation | Time (Min.) | Analysis costs | Instrument costs | Sample size | Risk of contamination | Precision |
|---|---|---|---|---|---|---|---|
| A | 3 Stunden | 1300 | 250 $ | 150 K$ | 0,2-1 g | medium | < 9% |
| B | 2 Stunden | 20 | 400 $ | 2 M$ | 1 mg | high | < 1% |
| C | 3 Stunden | 1300 | 250 $ | 150 K$ | 2-10 g | low | < 2% |
| D | 3 Minuten | 330 | 150 $ | 100 K$ | 5-15 g | low | < 3% |

**Table 2: Differences between methods A – D.**

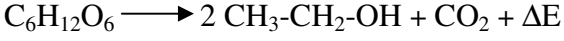
Other technologies, not using liquid scintillation technology, such as chromatographic or IR-spectrometric technologies can be used to identify and quantify ethanol or FAME but they can not distinguish between biogenic ethanol or FAME and synthetic, fossil ethanol or FAME. This can only be done with the help of scintillation technology or mass spectrometry. On the following pages we will discuss method D for the quantification of Biofuels.

**What kind of bio materials will be measured?**

In fuels ethanol, ETBE (Etyhl-tert-butylether) and MTBE (Methyl-tert-butylether) are the most common bio additives. In diesel fuel FAME (fetty acid methylester), RME (rape methylester), BTL (Biomass to liquid) and GTL (Gas to liquid) are the most often used bio additives.

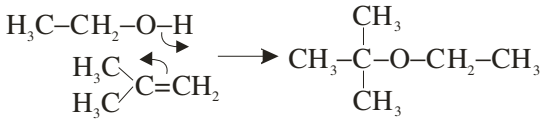
**How do we produce biofuels?**

In normal Otto fuel mainly bioethanol and ETBE is used. Bioethanol originates from the alcoholic fermentation of sugars:



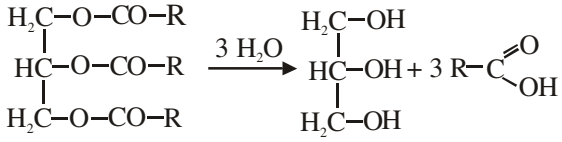
Sugars are mainly made out of sugar cane, sugar beet or especially in Germany from corn. Sugars are produced by enzymatic or acid induced cleavage from starch molecules.

ETBE will be produced from Isobuten and bioethanol via an addition reaction:

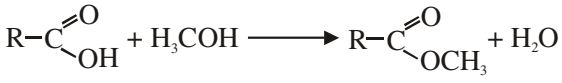


(EtOH + Isobuten) (ETBE)

Biodiesel mainly consists of FAME (Fatty acid methylester) produced from rape why it is also called RME (Rape methylester). The fatty acid which are available from rape can not directly be used for traditional engines in most cases, because their viscosity is very high. RME almost exclusively consists of fatty acid esters of glycerol. These tri-glycerides have to be cleaved in oil refineries where the cleaved fatty acid will be converted into the corresponding methylester.



Tri-glycerid ⟶ glycerol + fatty acid



Fatty acid + methanol ⟶ FAME

This chemical procedure is necessary because tri-glycerides show properties which are unwanted in engines.

Some tri-glycerides are very viscous or even solids (for example bovine tallow) and can especially not used at low temperatures in classical engines. Esters with multiple double bonds such as linol or linolenacid can be oxidized by air and tend to show radical polymerization.

**Principle of scintillation counting:**[9)]

Liquid scintillation counter measure the radioactivity via indirect measurement of light with the help of photo multipliers (PMT´s). The light is a results of an interaction between ionizing radiation and a so called cocktail[11)] which will be added to the sample. As you will see soon we need extremely sensitive instruments for the detection of natural radioactivity. The TriCarb 3170TR/SL uses in addition to coincidence technology and patented time resolved measurement technology[12, 13)] a guard-detector made out of bismuthgermanate (BGO). The combination of these technologies results in extremely high sensitivity due to a drastically reduction of background pulses without sacrificing counting efficiency. Figure 2 illustrates the TriCarb surround-guard-detector.

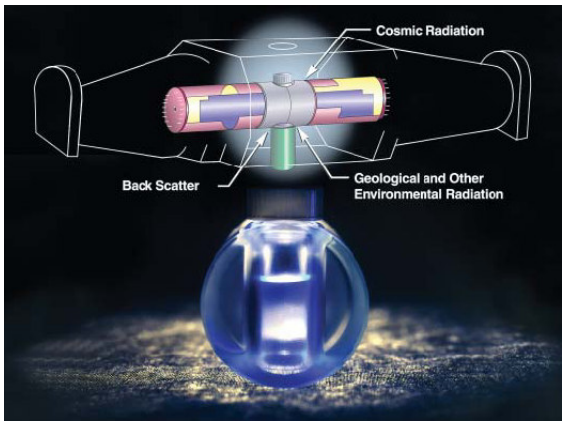



**Figure 2: BGO-detector in TriCarb 3170**

Another very sensitive instrument which can be used for this application is the Quantulus from PerkinElmer. This instrument uses very efficient lead shielding (630 Kg) in combination with an anti-coincidence circuit which also allows extremely low background values.

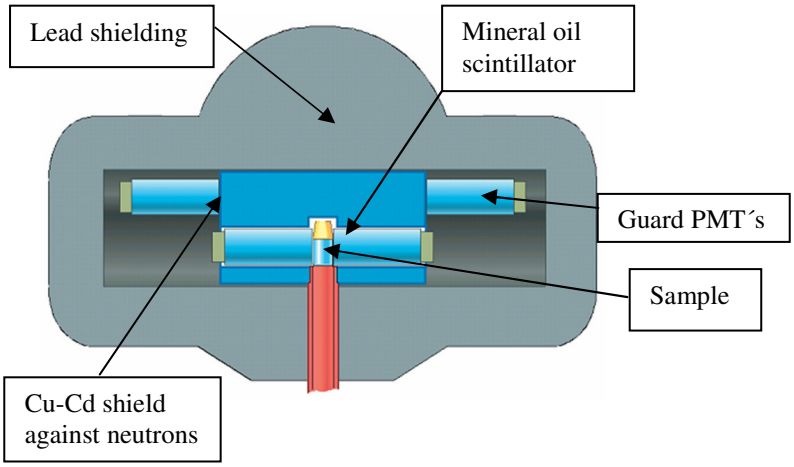



**Figure 3: Quantulus shielding and Guard-PMT´s**

Both systems offer high sensitivity and offer latest quench correction methods. The instrument that best suits your individual application should be determined during a discussion with you and one of PerkinElmer's specialists.

**What is the necessary sensitivity of the scintillation counter?**

To answer the question about the minimum sensitivity of a liquid scintillation counter for the measurement of biogenic samples we have to estimate the expected activity in such a sample. Because biodiesel and bioethanol are available in large quantities we should use a much sample as possible for the measurement in the LSC to increase the activity. With an optimized cocktail we should be able to use cocktail sample ratios of 1:1 (cocktail:sample) or even a slight excess of sample (possible with bioethanol) because the sample is of purely organic nature. Undiluted biodiesel can show a significant yellow colour but because currently maximum content in biodiesel for traditional engines is in the range of 5% the colour will be heavily diluted thus reducing colour quench significantly allowing direct measurement of these samples.

The purely organic nature of biodiesel allows the use of cocktails without emulsifying additives resulting in a better performance of the cocktail. A typical diesel fuel currently contains approximately 5% biodiesel. A measurement vial with 10g of diesel fuel contains approximately 0.5g biodiesel.

The carbon content in such a mixture is roughly 86% resulting in a carbon amount of biogenic material of 0.43g. This carbon contains mainly the non radioactive carbon isotope $^{12}C$ and only a very small amount of the radioactive isotope $^{14}C$. Among one billion $^{12}C$ nuclides we find less than one $^{14}C$ nuclide. In the sample of less than 1 gram of carbon (0.43g) which we want to investigate we have the unimaginable amount of less than one billionth of a gram $^{14}C$. Nevertheless we can detect even such small amounts of activity. In one gram carbon we have 14.62 decays in every minute (related to the current specific activity of natural carbon). This means that we have 6.3 decays per minute (6.3 DPM or 0.1 Bq) in our sample containing 0.43g carbon. We now know the approximate activity of our sample. What kind of scintillation counter do we need fort hese activities. We can use the DIN formulas to calculate the detection limit and the critical level of detection.[14)] To calculate the deteciton limit we need several values. The measurement time has a significant influence on the detection limit; we have to know the background of our system and the sample volume as well as the counting efficiency. From all these values we can determine the detection limit. In this application note we use formulas from DIN norm 25482. For details about counting statistics and error calculation please also read application note 25[14)].

Target: Measurement of a sample containing 6.3 DPM.

To check if our scintillation counter is suitable fort his method we first have to determine the critical detection limit:

$$g^* = \frac{k_{1-\alpha}}{E \cdot V}\sqrt{R_0 \cdot \left(\frac{1}{t_0}+\frac{1}{t_m}\right)}\ \text{Bq/L}$$

The sensitivity of the scintillation counter can be determined using the formula for the detection limit:

$$g = \frac{k_{1-\alpha}+k_{1-\beta}}{E \cdot V}\sqrt{R_0 \cdot \left(\frac{1}{t_0}+\frac{1}{t_m}\right)}\ \text{Bq/L}$$

The values $k_{1-\alpha}$ and $k_{1-\beta}$ include errors of 1. and 2. order. The values $t_0$ and $t_m$ are measurement times for background and sample. In case both counting times are identical the formulas can be simplified as follows for the critical level of detection:

$$\boldsymbol{g^* = \frac{k_{1-\alpha}}{E \cdot V}\sqrt{\left(\frac{2R_0}{t}\right)}}\ \text{Bq/L}$$

and for the detection limit:

$$\boldsymbol{g = \frac{k_{1-\alpha}+k_{1-\beta}}{E \cdot V}\sqrt{\left(\frac{2R_0}{t}\right)}}\ \text{Bq/L}$$

Biofuels can be measured in LSC´s without strong quench. Only samples containing FAME have to be diluted with excess of cocktail to reduce colour quench. Because no water is present in the samples we can use a pure organic cocktail such as Ultima Gold F resulting in very high counting efficiencies and high uptake capacities. A sample of 11ml diesel and 8 Ultima Gold F with a counting efficiency of 75% in a TriCarb 3170TR/SL with 1.5 CPM Background (0,025 CPS) and a measurement time of one hour (3600 Sekunden) and a $k_{1-\alpha}$ value of 3.0 and $k_{1-\beta}$ value of 1.645 will result in a critical level of detection of:

$$\mathbf{g^* = \frac{3}{0.75 \cdot 0.011}\sqrt{0.025 \cdot \left(\frac{1}{3600}+\frac{1}{3600}\right)}}$$

$$\mathbf{g^* = 1.36}\ \text{Bq/L}$$

The critical level of detection is 1.36 Bq/L which is equal to 0.08 DPM/ml or 0.9 DPM/vial. As we want to determine an activity of 6.3 DPM in our vial which is much more than the critical level, the LSC can be used for this method.

For the detection limit we can calculate:

$$\mathbf{g = \frac{4.645}{0.75 \cdot 0.011}\sqrt{\left(\frac{2 \cdot 0.025}{3600}\right)}}$$

g = 2.1 Bq/L

The detection limit is 2.1 Bq/L which is equal to 0.13 DPM/ml or 1.38 DPM/vial. Only higher activities can be detected which is the case with our sample.

Because some countries have lower tax for biofuels there is large interest in the accurate quantitative determination of biofuels. The statistical precision of the obtained results is therefore of major importance for this application.

**Experimental part:**
The following measurements have been done with a TriCarb 3170TR/SL or the Quantulus, both from PerkinElmer. The evaluation of spectral data has been done with the SpectraWorks evaluation software. The cocktail used was Ultima Gold F (PerkinElmer Art. Nr. 6013179) and the vials were High Performance Glas Vials, 20ml (PerkinElmer part no. 6000128 or 6000134) or Teflon coated plastic vials (6000477). If not mentioned otherwise 10ml cocktail and 10ml sample have been used for the measurement. At this amount of cocktail colour quench was significantly decreased in biodiesel samples. Samples with bioethanol only did not show any colour quench. Recent experiments showed that better results can be obtained with sample cocktail ratios of 12:8.
Figure 4 illustrates four LSC spectra. The measurement time was always 1200 minutes. Spectrum **(a)** is a background measurement, spectrum **(b)** is pure bioethanol, spectrum **(c)** is Ultimate diesel fuel without any biodiesel, and **(d)** is 100% FAME in Cocktail. In spectrum **(b)** we can easily see the excellent $^{14}C$ signal up to an energy of approximately 60 keV. The low energy shift is typical and due to the chemical quench of the alcohol.

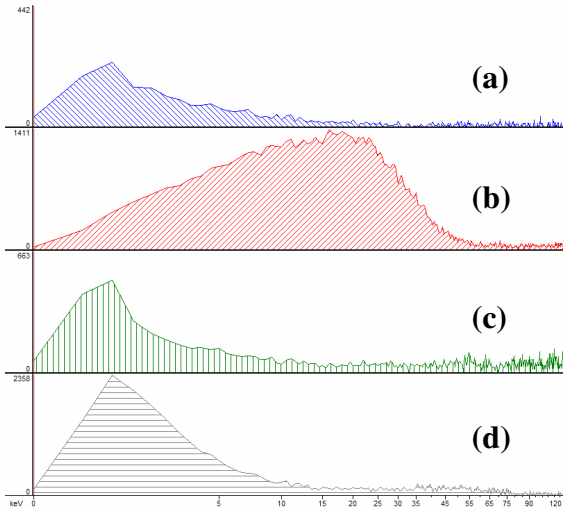



**Figure 4: Different fuels[7)]**

In spectrum (c) which is biodiesel free Ultimate fuel from Aral we do not see much activity as we expected. The activity is only slightly above background. This fuel is only based on fossil fuels and originally present activity should be decayed until today. As we could prove the signal between 0 and 4 keV is due to chemiluminescence. Keeping the samples overnight in the dark before starting counting could eliminate the luminescence.

| $^{14}C$ Counts in energy window 4-120 KeV | | |
|---|---|---|
| **Fuel** | **Counts** | **tSIE** |
| Background | 939 | - |
| Bioethanol | 46779 | 307 |
| Ultimate diesel | 1896 | 644 |
| FAME | 4798 | 15 |

**Table 3: Measurement results[7)]**

Heavy chemiluminescence could be detected with FAME samples. Cooling helped to reduce the level of luminescence in these samples. In $^{14}C$ samples chemiluminescence can be eliminated in most cases by reducing the energy window. Luminescence is a very low energy signal. Starting the measurement at 4 keV instead of 0 keV eliminates luminescence almost quantitatively without reducing the counting efficiency to much.
The lower „Counts“ value of FAME compared with bioethanol is mainly a result of the strong colour quench.

With a tSIE of 15 in this case a quantitative determinationn of the absolute activity is only possible with colour quench correction. In realistic fuel samples the amount of FAME usually does not exceed 10% resulting in a much lower colour quench. The following figure clearly shows that colour quench in FAME samples will be drastically reduced due to the increasing amount of cocktail.

Figure 5 illustrates the same samples (FAME) but in different ratios with cocktail. The bottom spectrum with only 0.5 ml FAME and 19.5 ml cocktail shows a significant shift of the spectrum to higher energies because quench has been eliminated due to the dilution with cocktail.

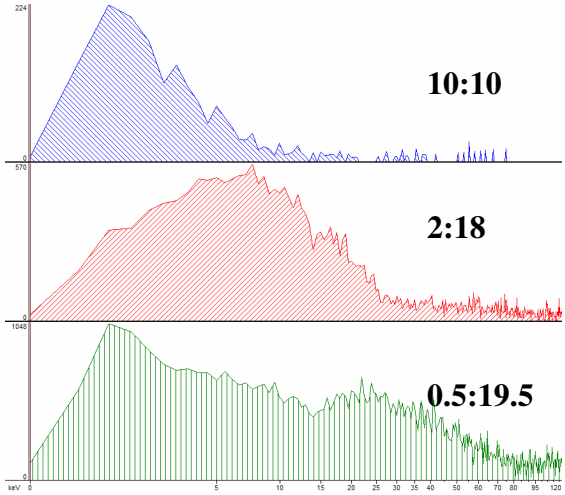



**Figure 5: FAME depending on dilution with Cocktail[7)]**

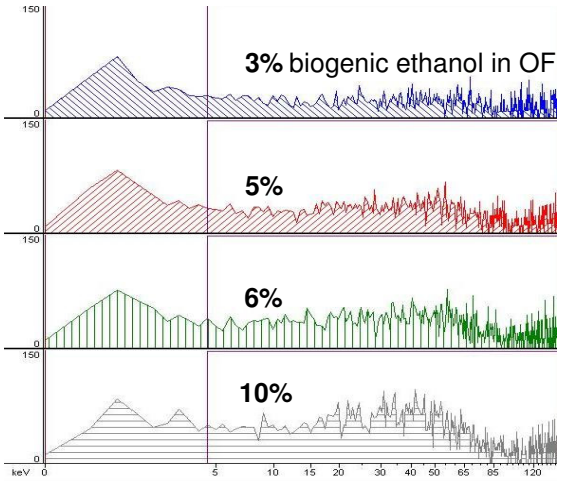



**Figure 6: $^{14}C$ spektra in Otto-fuel (OF) with different amounts of bioethanol.[7)]**

Figure 6 illustrates spectra of Otto-fuel with different amount of bioethanol. As shown in table 4 the quench parameter is almost constant in biofuel and with a value close to 500 the quench is only weak in contrast to the data for FAME in table 3. Colour quench is practically absent in bioethanol samples and therefore even CPM data allow a good determination of the amount of bioethanol in fuel.

| $^{14}C$ Counts in energy window 4-115 keV | | |
|---|---|---|
| **% Bioethanol** | **Total counts** | **tSIE** |
| 3 | 1420 | 527 |
| 5 | 1862 | 509 |
| 6 | 2024 | 501 |
| 10 | 2737 | 499 |

**Table 4: Results from OF with different bioethanol content[7)]**

The quench in bioethanol samples is exclusively due to chemical quench in the sample. This quench was more or less constant allowing the use of CPM measurements for a good correlation of measured counts and the amount of bioethanol in the fuel as illustrated in figure 7. We also measured bioethanol samples at the Finnanzlandesdirektion Vienna using the TriCarb 3170TR/SL. Here we used samples with even higher amount of bioethanol to investigate the influence of quench ot higher concentrations of alcohol. These measurements were performed with Teflon coated plastic vials. Usually plastic vials show better transmission for photons, lower reflection and lower background values due to the small amount of $^{40}K$.

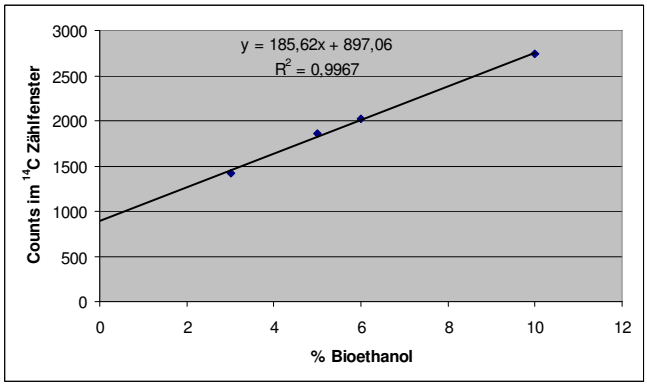



**Figure 7: Linearity of CPM measurements[7)]**

The optimum energy window was determined using the SpectraWorks software. For bioethanol in fuel we used an energy window ranging from 5-50 keV. Significant luminescence could be detected immediately after mixing ethanol and fuel. For this reason we left samples overnight in the dark inside the instrument before starting the measurement.

| CPM | CPM-Background | % Bioethanol | Time in minutes | Total counts | DPM | tSIE | Efficiency % |
|---|---|---|---|---|---|---|---|
| 1,1 | 0,0 | 0 | 80 | 87 | 2,3 | 474,65 | – |
| 4,8 | 3,7 | 10 | 480 | 2303 | 5,4 | 419,60 | 68,5 |
| 19,5 | 18,4 | 50 | 480 | 9355 | 26,6 | 360,36 | 69,2 |
| 36,9 | 35,8 | 100 | 480 | 17707 | 52,7 | 318,91 | 67,9 |

**Table 5: Measurement of bioethanol in the energy window from 5-50 keV[15)]**

As you can see the tSIE-value is decreasing significantly reflecting the increasing quench at higher concentrations of bioethanol. This however has almost no influence on the counting efficiency which only varies between 69.2 and 67.9 %. Therefore even CPM-measurements can be used to determine the amount of bioethanol in fuel. Figures 8 and 9 clearly indicate that in addition to DPM determinations simple CPM measurements can be used to quantify bioethanol in fuel. Thus the measurement of external standards can be avoided as long as no other quencher or colour is present.
This is of course impossible in the case of FAME because this sample shows strong yellow colour which may result in a strong decrease in counting efficiency. The use of bromine seems to reduce some colour of FAME samples as indicated by a first experiment.

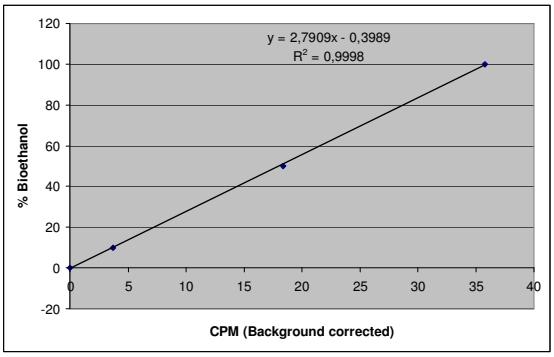



**Figure 8: Linearity of the CPM measurement[15)]**

Bleaching with oxidizing chemicals (which has to be done very carefully using explosion protection) or the use of active carbon does not result in significant colour reduction.

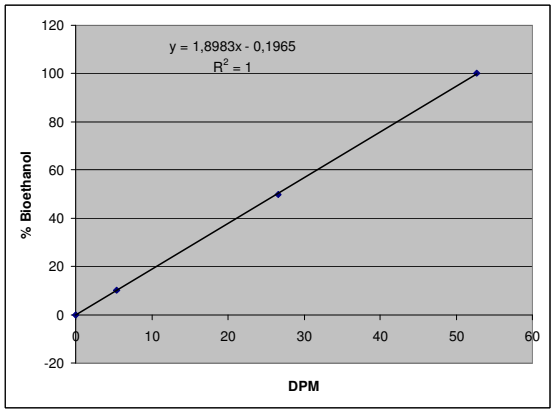



**Figure 9: Linearity of the DPM measurement[15)]**

Besides the use of the TriCarb 3170TR/SL we also used the Quantulus in our low level laboratory in Turku which is also a very sensitive instrument for the measurement of small biofuel components. As a result of this experiments a first paper has been published recently.[6)] Figure 10 illustrates the good correlation between the concentration of biomaterial and the obtained results. In this publication the authors also mention that biofuels can be mixtures of samples such as bioethanol and ETBE. In such a case calibration curves have to prepared for each component and it is necessary to know the exact composition of the fuel to do accurate quantifications.

The determination of the exact fuel composition can be done using methods such as GC-MS or NMR. It is also possible that components will be prepared from fossil and biogenic materials. For example ETBE can be prepared from bioethanol by addition reaction to fossil isobutene.

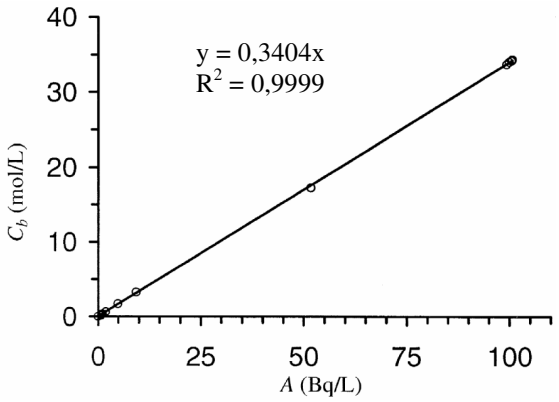



**Figure 10: Linearity of DPM measurements in the Quantulus[6)]**

Figure 10 illustrates the concentration of bioethanol in mol/L versus the activity. The measurement time in this case was 5.5 hours.

**Discussion of the results:**

The first measurements clearly indicate that the LSC technology especially using instruments such as the TriCarb 3170TR/SL and the Quantulus are superb instruments for the investigation of biogenic components in fuel. The quantification of biofuel is possible. Due to the low activities in these samples and measurement times in the order of 5 to 8 hours per sample special super low level scintillation counters are required.

For the future it would be helpful to find better ways to reduce the colour of biodiesel samples and still be able to do direct measurements of biofuel. Alternative LSC methods which are also available are sample combustion and the measurement of $CO_2$ converted into carbamate or the conversion of $CO_2$ to benzene as discussed above in Table 1.

## Literature


1. P. Cox, M. Chrisochoidis; *Official Journal of the European Union* **L123**, 42-46 (2003).
2. M. Stuiver, H. A. Pollach; *Radiocarbon* **19**, 355-363 (1977).
3. D. J. McWeeny, M. L. Bates; *J. Food Technology* **15**, 407-412 (1980).
4. I. U. Olsson; *Earth-Science Reviews* **4**, 203 (1968).
5. J. E. Noakes, G. Norton, R. Culp, M. Nigam, D. Dvoracek; LSC 2005, Advances in Liquid Scintillation Spectrometry, Proceedings of the 2005 International Liquid Scintillation Conference, Katowice, Poland, Edited by S. Chalupnik, F. Schönhofer, J. Noakes; Seite 259 (2007).
6. I. J. Dijs, E. van der Windt, L. Kaihola, K. van der Borg; *Radiocarbon* **48**, 315-323 (2006).
7. H. Blanke; C-14 Measurements in fuel, 3rd LSC User Meeting in Germany, part of the 222nd PTB seminar about determination of activity with the help of liquid scintillation technique, 21st Nov. 2006, Braunschweig.
8. F. Matiasovits, M. Sagaischek, R. Edler; Measurements of bioethanol in fuel with the TriCarb 3170TR/SL at the Finanzlandesdirektion Vienna, Technische Untersuchungsanstalt, Vordere Zollamtsstraße 5, 1030 Vienna, 23.-25. April 2007.
9. R. H. W. Edler, Eine Einführung in die Szintillationstechnik zur Messung von Radionukliden, in print (2007), ISBN-10: 3-00-020422-9, ISBN-13: 978-3-00-020422-7.
10. PerkinElmer LAS (Germany) GmbH, 63110 Rodgau-Jügesheim, LSC application note 1, Radio-Carbon dioxide ($^{14}CO_2$) Trapping and Counting, Mai 2005.
11. PerkinElmer LAS (Germany) GmbH, 63110 Rodgau-Jügesheim, LSC application note 16, Cocktails for Liquid Scintillation Counting, December 2006.
12. PerkinElmer LAS (Germany) GmbH, 63110 Rodgau-Jügesheim, LSC application note 11, Time Resolved Liquid Scintillation Counting (TR-LSC) in TriCarb Models from PerkinElmer LAS, July 2004.
13. PerkinElmer LAS (Germany) GmbH, 63110 Rodgau-Jügesheim, LSC applications note 15, TR-LSC with „Delay before Burst“, October 2006.
14. PerkinElmer LAS (Germany) GmbH, 63110 Rodgau-Jügesheim, LSC Application Note 25, Possible errors in measurements and basics of counting statistics for liquid scintillation counting“, (in print).
15. M. Sagaischek, F. Matiasovits, R. Edler, Bioethanol-measurments in the Finanzlandesdirektion Vienna, Vordere Zollamtsstraße 5, 1030 Vienna, 23.-25. April 2007.

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# Case Studies

PerkinElmer®
*For the Better*

# Helping BioEnergy of America LLC Make the "Best Biodiesel in America"

*Figure 1.* Monte Malone, BioEnergy of America's national sales manager with the PerkinElmer Clarus Gas Chromatograph.

As the world searches for new sources of fuel, maybe the answer to the problem could be found in every household kitchen. Wouldn't it be convenient if we could use everyday cooking oil instead of gasoline to run our cars? It may sound absurd, but it is slowly becoming a reality. A renewable and biodegradable fuel source called biodiesel, which can be made from vegetable oil, is rapidly gaining momentum around the world as an alternative fuel source for diesel engines. It is considered renewable, because it can be derived from plants, which produce oil from natural sunlight, water and air every year; and biodegradable, because unlike petroleum-based fuels, it breaks down into its natural components in the ground. Currently, the industry is producing approximately 300 million gallons of biodiesel per year, but with the anticipated demand, additional manufacturing plants are being built, which will increase production by another 600 million gallons per year.

## Benefits of biodiesel over fossil fuels

So what is commercially attractive about biodiesel, besides the fact that it is not a product of crude oil? Here is a list of the recognized benefits of biodiesel, which are all well-documented in the public domain:

- It requires about one third of the energy to produce 1 gallon of biodiesel compared to petroleum diesel.
- Biodiesel is extremely friendly to our environment, by reducing emissions of carbon monoxide, carbon dioxide (compared to the amount sequestered during the growing process), hydrocarbons and other particulate matter that causes respiratory damage.
- Another environmental attraction of biodiesel is that its sulfur content is less than 15 ppm, compared to 500 ppm for conventional S500 diesel fuel. This means that the emission of harmful sulfur dioxide, which contributes to acid rain, is significantly reduced.

- It also eliminates the cloud of dense, black smoke normally associated with diesel vehicles – in fact, the exhaust fumes from an engine running biodiesel smells like popcorn or French fries.
- Biodiesel also has better lubricating properties than regular diesel fuel because of its higher viscosity.

Its environmentally-friendly image has captured the attention of one of our most famous and controversial celebrities. Last year, you might have seen photographs of Willie Nelson on a nationwide concert tour. While Willie was traveling from venue to venue in his luxurious touring bus, one of the trucks in his ensemble was towing a tank of biodiesel, which was being used to refuel the bus. He is so enamored by this new fuel, which he discovered in Hawaii where he has a home, that he recently formed a company called Willie Nelson's Biodiesel. Their main product, called *BioWillie®,* which is predominantly made from soybeans, is being marketed directly to truck stops and gas stations.

## Biodiesel production

Biodiesel is produced by a chemical process known as transesterification, where a vegetable oil or animal fat is reacted under heat with an alcohol, in the presence of a catalyst. The products of the chemical reaction are an alkyl ester (commonly referred to as a biodiesel) and glycerin. The reacting components in the vegetable oil are actually mono-, di-, and triglycerides, which consist of long chains of carbon, hydrogen, and oxygen atoms called fatty acids. Soybean oil is the most common crop used in the U.S. for the production of biodiesel. It consists of pure triolein, which is a triglyceride where all three fatty acid chains are oleic acid. If triolein is reacted with methanol at 120 ˚F, using potassium hydroxide as a catalyst, the alkyl ester called methyl oleate will be formed together with the by-product, glycerin. Once the alkyl ester is separated from the glycerin and washed with water, it is ready for use. The commercial attraction is that the manufacturing process is very straightforward, doesn't require a huge financial investment and more importantly, the yield of alkyl ester in the chemical reaction is approximately 100%.

*Figure 2.* BioEnergy of America facilities based out of Denver, CO.

## BioEnergy of America

One of the leading new producers taking advantage of the explosion in demand for biodiesel is *BioEnergy of America,* LLC (Figures 1 and 2), based in Denver, CO. The company was founded in 2004 by several entrepreneurs, with a mission to make "the best and highest quality biodiesel in America." They began the venture by acquiring a small environmental company that cleaned asphalt out of railcars. Due to the favorable nature of the market conditions at the time, it only took them about 6 months to start production, mainly because they inherited the equipment, including a 400,000 gallon tank farm, rail access, transfer equipment, together with an existing laboratory and ancillary equipment. Their capacity is currently 20 million gallons of biodiesel per year, with plans to expand production to 100 million gallons by building several new facilities in the next few years.

Their main product is B100 (100%) biodiesel, which they currently sell to petroleum companies and fuel distribution organizations to make blends of biodiesel with petroleum-based diesel for commercial use. Although neat, undiluted biodiesel can be used in some diesel engines, the engine has to be modified in order to ensure "trouble-free", long-term use. It is therefore more usual for it to be blended with petroleum diesel. Biodiesel blends are typically denoted as representing the percentage of biodiesel contained in the blend. For example, B20 is a 20% biodiesel, 80% petroleum diesel mix. Many U.S. states are now passing legislation to mandate that all petroleum diesels for road use contain at least 2% biodiesel.

## Ensuring quality of biodiesel fuel

Unless the transesterification process is closely monitored and well controlled, biodiesel will most likely contain high levels of impurities. For that reason, it is absolutely essential that commercial grade biodiesel be supported by a rigorous quality control procedure. *BioEnergy of America* is therefore very proud

of the fact that their in-house QA/QC process ensures the highest quality product, by adopting the ASTM D-6751 standard method for the production of B100 biodiesel. This method covers the analytical methodology and specification for biodiesel that is used for blending purposes with petroleum diesel fuels. This Standard, which is comprised of 14 separate physical and chemical tests, including flash point, viscosity, cloud point, moisture, sulfur and glycerin content, guarantees that all biodiesel manufactured for use in diesel engines conforms to the highest purity standards. This means that the manufacturing process is under tight control and produces a product that has no adverse effects on the engine, is going to run with no long term degradation of the engine components, is free of contamination and is not going to pollute the air with toxic gases or particulates. It is important to emphasize that in order for biodiesel to be registered as a fuel, it must meet U.S. EPA health effect regulations as defined by 40 CFR, Part 79. For that reason, the National Biodiesel Board (NBB) has completed the required health effects testing on behalf of the industry and has deemed that an alkyl ester can only be called biodiesel if it meets the ASTM D-6751 specifications.

## Gas chromatography

Of the 14 individual ASTM test methods that cover the analysis and specification of biodiesel, probably the most important one with regard to the manufacturing process is ASTM D-6584 – A Test Method for the Determination of Free and Total Glycerin in B100 Biodiesel Methyl Esters by Gas Chromatography (GC) Using Flame Ionization Detection (FID). Measuring the level of free glycerin and any unreacted mono-, di- and tri-glycerides in the biodiesel indicates how efficiently the transesterification reaction is proceeding. Ideally, all the vegetable oil will react with the methanol and be converted to the methyl ester. Analyzing the sample using this GC method will give an indication as to whether there are any unreacted triglycerides in the final product as well as any traces of free glycerin.

For this crucial analysis and to carry out other purity tests, *BioEnergy of America* utilizes a PerkinElmer® Clarus® Gas Chromatograph (Figure 3), with an 82-vial autosampler and dual FID detectors. The instrument is an integral part of their QA/QC process to ensure that biodiesel and other related products meet ASTM specifications. They run approximately 100 samples per week using this approach, comprising many different types of analyses, including the measurement of contaminants in incoming raw materials and monitoring the purity of various manufacturing process streams. In addition to this QC function, the GC system is also used as a troubleshooting research tool to support their biodiesel production process.

Clearly, the Clarus GC is critical to the philosophy of high quality at *BioEnergy of America,* as explained by Geoff Brown, the principal scientist in charge of the QA/QC process, "We utilize several proprietary manufacturing processes and rely on GC analysis to monitor performance and quality of both the process and effluent streams. The Clarus GC and, in particular, its high up-time, is therefore vital to our production of ASTM-specification grade biodiesel".

"We chose PerkinElmer because of their product innovation, intelligent software, recognized customer support and high reliability at an economic price," said Monte Malone, Vice President of Sales, Western Region for *BioEnergy of America.*

As they plan for expansion with new production plants, we are excited to be their partner in an industry which is going to see unprecedented growth in the next few years – clearly an industry where quality is going to define the marketplace.

We'd like to leave the last word to Tom Davanzo, the President of the company, who fully understands the business implications of high quality in this potentially huge new market: – "…our proprietary manufacturing process and quality assurance/control procedures allows us to make the claim that *BioEnergy of America* produces the best soy-based methyl ester biodiesel in the USA".

*Figure 3.* Clarus Gas Chromatograph.

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**PerkinElmer Instrumentation Goes the Distance to Ensure Fuel Quality in Indy Racing**

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Testing and certifying fuel for purity and consistency ensures that every car competing in the 16-race 2008 IndyCar® Series schedule, including the Indianapolis 500, is on a level playing field. Fuel testing is one of more than 130 check points on a race car – from head and neck restraints to tire pressure and engine components – all to ensure that the first car to cross the finish line got there fairly.

Parked in the garage area of each IndyCar Series track is a sophisticated fuel analysis laboratory. Inside, PerkinElmer scientists analyze and report their findings of fuel composition to certify it is race-ready. PerkinElmer has been testing and certifying racing fuel since the inception of the IndyCar Series in 1996.

IndyCar Series regulations stipulate that the fuel must be 100 percent fuel-grade ethanol, which is a blend of 98 percent ethanol denatured with 2 percent 98 octane unleaded gasoline. The IndyCar Series, guided by PerkinElmer experts, migrated to fuel-grade ethanol in 2007 to achieve more efficient engine performance and contribute to the lessening of dependence on oil-based fuel.

Brett Boyer, Senior Service Engineer, PerkinElmer Life and Analytical Sciences, runs the onsite testing lab. PerkinElmer is the official instrument supplier and fuel certification partner for the Indianapolis 500 and the IndyCar

Series. Boyer and his PerkinElmer team test and certify fuel for purity and consistency from the source of the fuel and through the supply chain to onsite on race day at IndyCar Series races in the United States.

For many years, 100 percent methanol was used in the high-performance race car engines. In 2006, the IndyCar Series introduced a blend of 90 percent methanol and 10 percent ethanol as a transition fuel before full incorporation of ethanol. The IndyCar Series' conversion to the renewable fuel was introduced by the late Paul Dana, a driver who was killed in 2006 during a practice session, and was supported by the Ethanol Promotion and Information Council (EPIC), a non-profit alliance of ethanol industry leaders.

PerkinElmer's technical assistance was critical to ensure that the migration to ethanol was not at the expense of engine performance and safety.

PerkinElmer tests use its Clarus® 500 gas chromatograph (GC) controlled by a PerkinElmer TotalChrom® chromatography data system for collecting, processing and reporting data. The Clarus 500 GC separates the fuel to identify additives that may give one car a competitive advantage over others. The testing takes approximately 5 minutes and can detect impurities down to concentrations of 0.10 percent.

Once the blend is analyzed there, it is shipped for testing and storage at Superior Oil in Indianapolis, Ind. Next, it is shipped by tanker truck to IndyCar Series tracks across the country. Superior Oil uses five PerkinElmer Clarus 500 GCs in Indianapolis to conduct the initial fuel tests. To ensure quality control, PerkinElmer tests the fuel composition after every delivery.

Once fuel arrives at a racetrack, PerkinElmer experts test it before and after qualification rounds and after each race. The Clarus 500 can test an average of 25 samples from all the cars entered in each race, along with the top five cars after the race. Fuel from the fuel tanker is the baseline sample against which the samples are compared. PerkinElmer engineers run the analyses the night prior to a race. On race day, they simply call up the results and overlay them with the baseline sample to determine if there are any abnormalities. For the Indy 500, nearly 100 samples are tested for the two weekends of qualifications, and the top 12 finishers are tested after the “Greatest Spectacle in Racing.”

Kevin Blanch, Technical Director for the IndyCar Series, relies on PerkinElmer for testing the fuel in each car a minimum of three times over the course of a racing weekend. The analyses yielded positive results upon occasion.

To determine the ideal fuel blend, PerkinElmer and IndyCar Series engineers tested a range of options using a dynamometer to gauge the BTUs and RPMs. The transition required minor calibration changes only since the IndyCar Series cars were already running methanol, another alcohol-based fuel. To further protect engine integrity, a Honda engineer is assigned to and stationed with every car to troubleshoot engine issues. Series officials also decided to reduce the size of the fuel cell from 30 gallons to 22 gallons, because ethanol is more fuel efficient than methanol.

The composition of engine oil also can impact performance. PerkinElmer analyzes the race cars' engine lubricants for contaminants and conformance to IndyCar Series requirements. The PerkinElmer Spectrum ™ 100 infrared spectrometer is used for both the IndyCar Series and Indy Pro Series and provides instantaneous results to ensure contaminant-free performance.

With the conversion to ethanol, the IndyCar Series offers fans and drivers alike all of the fast-paced excitement they've come to enjoy – and the added benefit of a safe, renewable energy source that ensures that the IndyCar Series is on track for a safe, clean and environmentally sound future.

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# Articles and Publications

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FEBRUARY 2006

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## Biodiesel: a renewable and biodegradable fuel

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# Biodiesel: a renewable and biodegradable fuel

## New US specification ensures product identity and quality for biodiesel

**M. BOWMAN, D. HILLIGOSS** and **S. RASMUSSEN,** PerkinElmer Life and Analytical Sciences, Shelton, Connecticut, and **R. THOMAS,** Scientific Solutions, Washington, DC

Biodiesel is a renewable and biodegradable fuel refined from vegetable oil (or animal fat). It is rapidly gaining momentum in the US as an alternative fuel source for diesel engines. Currently, the industry is producing approximately 300 million gallons per year (gpy) of biodiesel, but, with the anticipated demand, additional manufacturing plants are being built. This will increase production by another 600 million gpy in the near future.

What is commercially attractive about biodiesel, besides not being refined from crude oil? Compared to petroleum diesel, biodiesel is environmentally friendly and is government mandated. It reduces carbon monoxide (CO), carbon dioxide ($CO_2$), sulfur dioxide ($SO_2$), hydrocarbons (HC) and other particulate matter emissions that cause respiratory damage. Biodiesel also eliminates the cloud of dense, black smoke normally associated with diesel vehicles. The exhaust fumes from an engine running biodiesel smells like popcorn or french fries. It also has better lubricity than diesel fuel because of its higher viscosity.

Biodiesel's environmentally friendly image has captured the attention of one of the US's most famous and controversial celebrities. While Willie Nelson travels from venue to venue in his luxurious touring bus, one of the trucks in his ensemble tows a tank of biodiesel, which refuels the bus, pictured here. Nelson is so enamored by this new fuel that he formed a bioenergy company—Willie Nelson's Biodiesel. The main product, *BioWillie,* is predominantly refined from soybeans and is being marketed directly to US truck stops and gas stations.

**Benefits.** Some of the advantages that biodiesel has over petroleum-based diesel include:

**Requires less energy.** The fossil fuel energy required to produce biodiesel from soybean oil is only 30% of the energy contained in one gallon of the fuel. In other words, approximately 3.2 units of fuel energy are generated from biodiesel for every unit of fossil energy used to produce the fuel. That estimate includes the energy used in diesel farm equipment and transportation vehicles (trucks, locomotives); fossil fuels used to produce fertilizers and pesticides; fossil fuels used to produce steam and electricity; and methanol used in the manufacturing process.

**Harmful emissions reduction.** When biodiesel displaces petroleum, it reduces levels of global warming gases such as $CO_2$. As plants like soybeans grow, they take $CO_2$ from the air to make the stems, roots, leaves and seeds. After the oil is extracted from soybeans, it is refined into biodiesel and, when burned, produces $CO_2$ and other emissions, which are returned to the atmosphere. However, this cycle does not add to the $CO_2$ level in the air because the next soybean crop will reuse the $CO_2$ to grow.

Another important environmental factor is that biodiesel reduces tailpipe particulate matter (PM), HC and CO emissions. These benefits occur because biodiesel contains 11% oxygen ($O_2$) by weight. The presence of $O_2$ allows the fuel to burn more completely, resulting in fewer emissions from unburned fuel. This same principle also reduces air toxicity, which is associated with the unburned or partially burned HC and PM emissions. Testing has shown that PM, HC and CO reductions are independent of the vegetable oil used to make biodiesel. This has been confirmed by the EPA, which reviewed 80 biodiesel emission tests and concluded that the benefits are real and predictable over a wide range of biodiesel blends.

**Human health.** It is well-documented that many PM and HC emissions from petroleum diesel fuel combustion are toxic and suspected of causing cancer and other life-threatening diseases. Using biodiesel can eliminate a significant number of these toxic components. Biodiesels' positive impact on air toxicity is supported by numerous studies, including the Bureau of Mines Center for Diesel Research (BMCDR), The Department of Energy (DOE) and Southwest Research Institute (SRI). The National Biodiesel Board (NBD) also conducted Tier I and Tier II health effect studies under "The Clean Air Act" that also support these claims. Recently, the Department of Labor's Mining Safety Health Administration (MSHA) tested and approved using biodiesel in underground mining equipment where workers are exposed to high levels of diesel exhaust.

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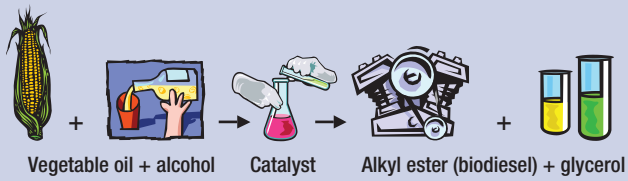


**FIG. 1** Simplified transesterfication of vegetable oil into biodiesel (alkyl ester).

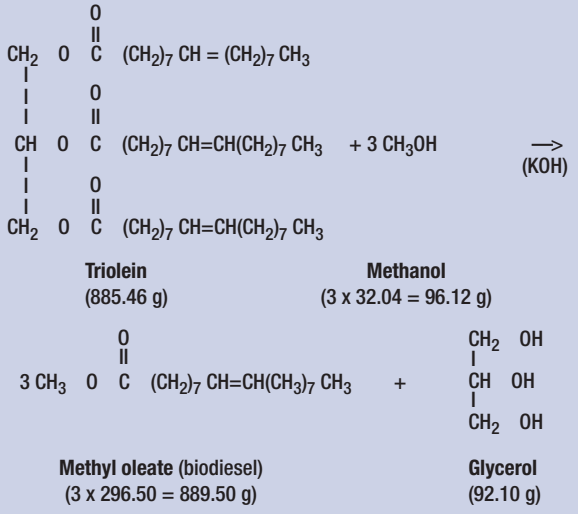


**FIG. 2** Methyl oleate (biodiesel) production from soybean oil.

**Low sulfur content.** Currently, the sulfur specification for petroleum-based diesel fuel is less than 500 parts per million (ppm). However, by the end of 2006, all US highway diesel has to contain less than 15-ppm sulfur. Most biodiesel fuels being manufactured today contain less than 15-ppm sulfur and some have levels that are too low to measure.

**Improved lubricity.** Engine manufacturers depend on good lubrication to keep moving parts, such as fuel pumps, from wearing prematurely. Biodiesel is approximately twice as viscous as petroleum diesel and therefore has better lubricating properties. This is an extremely important property when biodiesel is blended with ultra-low-sulfur diesel, which is known to be a poor lubricant. Even the lubrication properties of dry fuels such as kerosine can be improved by using 2% biodiesel.

**Implementation is seamless.** Probably the biggest benefit to using biodiesel is that it is easy to use. No new equipment is necessary and conventional diesel engines can seamlessly run up to 20% biodiesel blends. However, minor modifications to the engine are required to run neat, undiluted biodiesel. Biodiesel/petroleum diesel blends can also be stored in diesel fuel tanks and pumped with diesel equipment.

**Production.** Biodiesel is refined by transesterfication, where a vegetable oil or animal fat is reacted under heat with an alcohol, in the presence of a catalyst. The chemical reaction products are an alkyl ester, commonly referred to as a biodiesel and glycerol (Fig. 1).

The reacting components in vegetable oil are mono-, di- and triglycerides, consisting of long chains of carbon and hydrogen atoms or fatty acids. For example, soybean oil consists of pure triolein, which is a triglyceride where all three fatty acid chains are oleic acid. If triolein is reacted with methanol at 120°F, using potassium hydroxide as a catalyst, the alkyl ester called methyl oleate will be formed together with glycerol ($C_3H_8O_3$) as a by-product (Fig. 2).

The biodiesel yield from this chemical reaction is in the order of 100%. Thus, a unit weight of vegetable oil will produce the same unit weight of biodiesel. The process is so straight-forward that many people are making biodiesel in their garages at home for only a few thousand dollars investment. Bulk cooking oil can be purchased from a restaurant wholesale supplier; potassium hydroxide can be obtained from a hardware store, while alcohol can be purchased from a racetrack or chemical supplier. Once the chemicals are mixed at the right temperature and allowed to stand for a couple of hours, the mixture will separate into two distinct layers. The biodiesel (alkyl ester) will form at the top while the glycerol will settle to the bottom. Once the alkyl ester is separated from the glycerol and washed with water, it is ready to be used in a diesel engine.

However, it must be emphasized that, even though the transesterfication process is relatively straightforward, homemade biodiesel is not going to generate the highest-quality product. It will most probably contain impurities like residual alcohol, moisture, unreacted vegetable oil (triglycerides), incompletely reacted mono- and diglycerides, free fatty acids and trace metals from the catalyst.

Commercial manufacturers must ensure that the manufacturing process quality is well-controlled in order to produce bulk biodiesel for blending with petroleum products. For that reason, all commercial-grade biodiesel has to conform to the American Society of Testing Materials International's ASTM D6751 specification if it is going to be used in diesel engines.

Although neat, undiluted biodiesel can be used in most diesel engines, the engine has to be modified in order to ensure trouble-free, long-term use. It is therefore more usual for it to be blended in small quantities with petroleum diesel. Biodiesel blends are

**TABLE 1. Analytical test method and specification limit as defined by ASTM D-6751 for S15 biodiesel (Note: S500 grade differs only in sulfur content which is set at 500 ppm.)**

| Property/analyte | ASTM test methodology | Biodiesel (S15) specification limits |
|---|---|---|
| Flash point | D-93 | 130°C (min) |
| Water and sediment | D-2709 | 0.050% by volume |
| Kinematic viscosity (40°C) | D-445 | 1.9–6.0 $mm^2/s$ |
| Sulfated ash | D-874 | 0.020% by mass (max) |
| Sulfur | D-5453 | 15 ppm (500 ppm for S500) |
| Copper strip corrosion | D-130 | Nº 3 (max) |
| Cetane number | D-613 | 47 (min) |
| Cloud point | D-2500 | Report result in °C |
| Carbon residue | D-4530 | 0.050% by mass |
| Acid number | D-664 | 0.080 mg KOH/g (max) |
| Free glycerol | D-6584 | 0.020% by mass |
| Total glycerol | D-6584 | 0.240% by mass |
| Phosphorus | D-4951 | 0.001% by mass (max) |
| Distillation temperature | D-1160 | 360°C (max) |

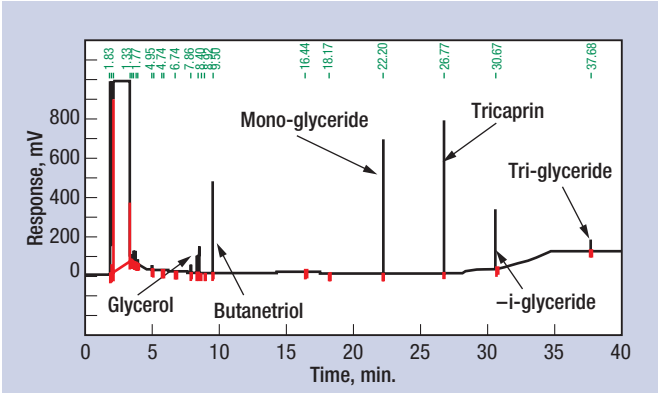


**FIG. 3** Chromatographic display of a calibration mixture of glycerol, mono-, di- and triglyceride and the internal standards butanetriol and tricaprin—generated using ASTM Method D-6584 (see Table 3 for concentration levels).

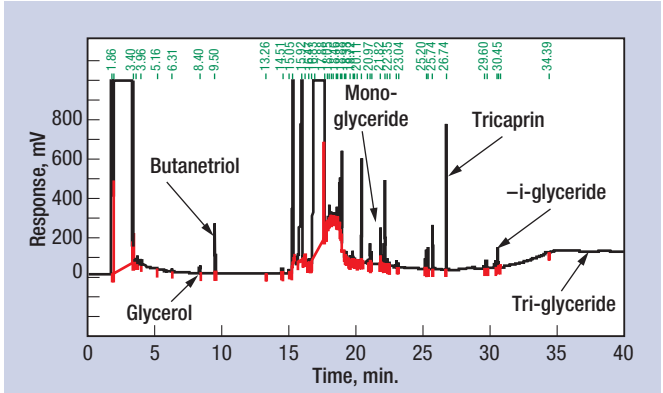


**FIG. 4** Chromatogram of a biodiesel sample with low levels of glycerol, mono and diglycerides, but no triglyceride—generated using ASTM Method D-6584.

typically denoted as "BXX" with "XX" representing the percentage of biodiesel contained in the blend. For example, B20 is a 20% biodiesel, 80% petroleum diesel mix. Many states are now passing legislation to mandate that all petroleum diesels for road use contain at least 2% biodiesel.

**Standard specification.** The National Biodiesel Board has adopted ASTM D-6751 as the standard to produce B100 biodiesel. In fact, a fuel cannot technically be called a biodiesel unless it meets the specifications set down in D-6751. The method covers the analytical methodology and specification for biodiesel grades S15 and S500 (variable sulfur content) that are used as blend components in low- and high-sulfur petroleum diesel fuels. The analytical methodology for each analyte and its specifications limits are summarized in Table 1.

This standard, which is comprised of both physical and chemical tests, is meant to guarantee that all biodiesel manufactured for use as a blend for diesel engines conforms to a purity standard. This ensures that the refining process is under control and produces a product that has no adverse effects on the engine, is going to run with no long-term engine components degradation, is contaminate free and is not going to pollute the air with any toxic gases or particulates.

D-6751, which was originally adopted as a standard by the ASTM committee on Petroleum Products and Lubricants in 2003, is actually made up of a compendium of 14 ASTM standard methods, and references an additional 21 other methods. Some of these test methods include:

• **Flash point** using D-93—A closed-cup test method: This is an indicator of the level of unreacted alcohol remaining in the fuel.

• **Viscosity** using D-445—A dynamic viscosity test method: Too high or too low a viscosity can result in power loss due to inefficiency in the injection pump.

• **Sulfur** using D-5453—An ultraviolet (UV) fluorescence method: Sulfur degrades engine wear by leaving deposits on engine components. It also impacts emission-control systems performance.

• **Acid number** using D-664—A potentiometric titration test method: This is used to indicate the level of free fatty acids or processing acids in biodiesel.

• **Phosphorus** using D-4951—An inductively coupled plasma atomic emission spectrometry (ICP-AES) test method: High levels of phosphorus have been shown to damage catalytic converters used in emission control systems. Note: Because ICP-AES is a rapid, multi-element technique, many labs are also determining calcium, magnesium, sodium and potassium using this method, to determine if the biodiesel contains any trace metal contamination derived from the catalyst and other material.

**TABLE 2. GC operating conditions to determine free and total glycerin in biodiesel**

| Sample size: 1 µL | | |
|---|---|---|
| **Column temperature program** | | |
| Initial temperature | 50°C | Hold for 1 minute |
| Rate 1 | 180°C at 15°C /min | |
| Rate 2 | 230°C at 7°C /min | |
| Rate 3 | 380°C at 30°C/min | Hold for 10 minutes |
| **Detector** | | |
| Type | FID | |
| Temperature | 380°C | |
| **Carrier gas** | | |
| Type | Hydrogen or helium | |
| Flow rate | 3 mL/min | Measured at 50°C |

• **Free glycerol** using D-6584—A gas chromatography (GC) test method: Free glycerol, which is a by-product of the transesterfication process, causes injector deposits, which can clog the fuel system. It can also build up in the bottom of storage and fuel tanks.

• **Total glycerol** using D-6584—A GC test method: This measures the level of free glycerin plus any unreacted oil or fats (mono-, di- or triglycerides) in the biodiesel. These unreacted glycerides can cause injector deposits and may adversely affect cold-weather operation.

Of all the individual ASTM test methods that cover biodiesel analysis and specification, probably the most important with regard to monitoring the actual refining process is ASTM D-6584—determining free and total glycerin in B-100 biodiesel methyl esters by GC. Measuring the level of free glycerol and any unreacted mono-, di- or triglycerides in biodiesel will indicate how efficient the transesterfication reaction is proceeding. Ideally, all the vegetable oil will react with the methanol and be converted

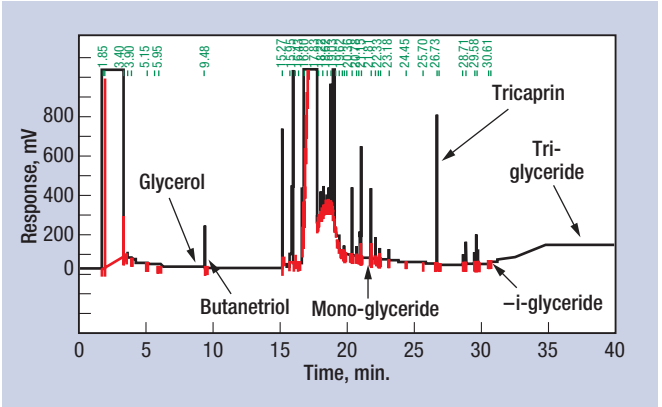


**FIG. 5** Chromatogram of a second biodiesel sample showing no glycerol or triglyceride peaks, but very small amounts of diglyceride and a high level of monoglyceride—generated using ASTM Method D-6584.

**TABLE 3. Concentration levels of the four analyte components and two internal standards seen in the chromatographic display shown in Fig. 3**

| Component | Concentration (µg) |
|---|---|
| Glycerol | 25 |
| Monoglyceride | 200 |
| Diglyceride | 500 |
| Triglyceride | 200 |
| Butanetriol | 1,000 |
| Tricaprin | 800 |

to the methyl ester. Analyzing the sample using this GC method will give an indication if there are any unreacted triglycerides in the final product as well as any traces of free glycerol.

**ASTM D-6584.** This describes a method for quantitatively determining free and total glycerol in B-100 methyl esters (biodiesel) by GC using flame ionization detection (FID) technology. The detection range for this method is 0.005%–0.05% for free glycerol and 0.5%–0.5% for total glycerol. The sample is first derivatized with a silyating agent and then injected into an open tubular GC column packed with a 5% phenylpolydimethylsiloxane.

Calibration is achieved with two internal standards (butanetriol and tricaprin) and four reference materials. Mono-, di- and triglycerides are determined by comparison with mono-olein, di-olein and tri-olein, respectively. Conversion factors are then applied to the results for mono-, di- and triglycerides to calculate the sample's bonded glycerol content. The total glycerol represents the sum of the free and bonded glycerol.

**Operating conditions.** One microliter (1µL) of sample is injected onto the column, which is then heated to 50°C. Initially, the GC oven is ramped up to 180°C at 15°C/min. It is then increased to 230°C at 7°C/min. Finally, it is ramped to 380°C at 30°C/min and held there for 10 minutes. The resulting chromatographic peaks are driven off the column using hydrogen or helium carrier gas at 3 mL/min and detected using an FID (Table 2).

The eluting peaks are then identified and quantified by comparing retention times and peak areas with the calibration standards, and the relative peak intensities and masses of the internal standards. Typical chromatograms for a calibration standard and two biodiesel samples using ASTM Method D-6584 are shown in Figs. 3, 4 and 5, respectively. Fig. 3 represents a calibration mixture spectral display of glycerol, mono-, di- and triglycerides (as mono-, di- and tri-olein) and the internal standards butanetriol and tricaprin. The x-axis shows retention time, while peak intensity is shown on the y-axis. Concentration values for all six components are summarized in Table 3.

It can be seen that the glycerol retention time is in the order of 8.5 min, while the mono-, di- and triglycerides are seen at 21.8 min, 30.7 min and 37.7 min, respectively. As expected, strong spectral signals are also seen for butanetriol and tricaprin at 9.5 min and 26.8 min, respectively. Fig. 4 identifies a biodiesel sample with low levels of glycerol, mono- and diglycerides, but no triglyceride, whereas Fig. 5 shows that the second sample contains no glycerol or triglyceride, a very small amount of diglyceride and a high level of monoglyceride. Both these biodiesel samples met the free and total glycerol specifications shown in Table 1.

**The final analysis.** Biodiesel represents a small but tangible way of breaking total dependence on fossil fuels. It is relatively straight-forward to produce and can be blended with petroleum diesel with no modifications to the engine. It is environmentally friendly because its combustion emission gases are nontoxic to the atmosphere—and it is also extremely biodegradable, posing no long-term health problems.

However, the relatively simple and inexpensive production process could also prove to be a disadvantage, because it will attract many commercial manufacturers. It is therefore absolutely essential that, if biodiesel is going to compete with fossil fuels, it must be manufactured to the highest purity standards. The only way this can be guaranteed is to ensure that the process is well-controlled by manufacturing to strict quality specifications like ASTM D-6751. There is no question that, by using sensitive instrumental techniques like GC, ICP-AES, UV fluorescence spectrometry and other traditional technology to monitor both the physical and chemical properties, it will help to enhance biodiesel's reputation as a high-quality, environmentally safe viable alternative to petroleum-based fuels. **HP**

**Matt Bowman** spent 12 years in the industry focused on instrumentation, process optimization and quality improvement. He joined PerkinElmer LAS in 2004 as a business development specialist and was recently promoted to global technical sales development manager. His key responsibilities are working with chemical, petrochemical and refinery customers to find innovative solutions for their toughest problems.

**David Hilligoss** is the business development manager for new and used oils business at PerkinElmer LAS. He has been with PerkinElmer for 29 years in numerous field application and support positions in the analytical instruments business. Mr. Hilligoss's current responsibilities are working with the lubricants industry in understanding their needs to develop better applications and instrumentation.

**Sandra Rasmussen** is the business unit manager for the gas chromatography business at PerkinElmer LAS and has over 20 years experience in the analytical instruments and biotechnology industry. She joined PerkinElmer in 1989 and has held senior roles in technology, marketing and business management throughout her career there.

**Robert Thomas** runs his own scientific writing company, Scientific Solutions. He has written over 50 articles/papers covering a wide variety of topics including analytical chemistry, spectroscopy, industrial process monitoring, biotechnology and life science subject matter. Mr. Thomas recently published a textbook on trace metals' analysis entitled, *Practical Guide to Inductively Coupled Plasma Mass Spectrometry (ICP-MS)* and he can be contacted on his Website at: www.scientificsolutions1.com.

## PerkinElmer - your partner in Biodiesel analysis

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*Special Feature* by Bernard Tulsi

# Biofuel Production Ups Demand for Analysis Instruments

*Part 2—Smarter Instrumentation Requires Better Training, Service and Support*

"New construction of bioethanol and biodiesel plants is continuing at a vigorous pace with laboratory services needing to match this growth," says Jim Mott, Ph.D., senior technical support specialist with Shimadzu Scientific Instruments. Dr. Mott adds, "The need to do basic instrumental analysis at biofuel production laboratories will continue to press the need for more analytical equipment."

An impressive repertoire of analytical instrumentation built on enabling techniques like gas and liquid chromatography, infrared (IR), and titration methodologies forms the nucleus of the test and measurement arsenal now deployed in the biofuels industry.

Several analytical-tool developers and marketers now serve the "hydrocarbon processing" sector, which encompasses biofuels, often drawing on expertise acquired from a long, fruitful and ongoing association with the petroleum fuels industry. Oil refineries buy about $1 billion in laboratory instrumentation annually, including some 20% of all the gas chromatographs made in the world.

"Bioethanol, based on the number of gallons produced per year, is a bigger industry than biodiesel. The emphasis in the United States is on bioethanol while in Europe it is biodiesel," says Dave Armstrong, chemical and semiconductor marketing leader with PerkinElmer.

"There tends to be more and different tests on biodiesel than on bioethanol," he adds.

Bioethanol analysis makes extensive use of liquid and gas chromatography. Liquid chromatography is primarily used to measure the amount of unfermented sugars from feedstock, such as corn or sugar cane, present in the fermentation vats at different time points during the transformation to ethanol.

A significant amount of the fuel producers' capital is tied up in the containers and in the process, which can go on for weeks, according to Armstrong. "So fuel manufacturers want to gauge the rate of fermentation carefully to ensure the intended level of sugar has been converted to ethanol.

"Once this happens, they distill the ethanol solution yielded during the fermentation process and then use gas chromatography to measure the purity level of the ethanol," says Armstrong.

Gas chromatography is also used

**Read more on page 11**

*Featured New Product*

## Trace Moisture Analyzer for Ultra-High Purity Gases

*Mini-Cavity Ring-Down Spectroscopy Analyzer Covers Wide Dynamic Range*


Tiger Optics LLC has introduced the HALO+™, a mini-Cavity Ring-Down spectroscopy analyzer capable of measuring at parts-per-trillion (PPT) levels. The new analyzer addresses the heretofore-unmet need for fast, accurate, calibration-free measurement of moisture in the parts-per-trillion (PPT) to parts-per-million (PPM) range. Companies in industries including semiconductor fabrication, laboratory calibration and industrial

*continued on page 12*

### Survey Says...

***When asked what percentage of their laboratory purchases are made through distributors vs. vendors, our readers report:***

| | |
|---|---|
| 0 – 20% of purchases are made through distributors | 17.8% |
| 21 – 40% of purchases are made through distributors | 22.2% |
| 61 – 80% of purchases are made through distributors | 21.8% |
| 81 – 100% of purchases are made through distributors | 12.3% |

*Results are based on an E-mail survey conducted in May 2007.*

*continued from front cover*

by biodiesel manufacturers to measure the amount of glycerin in the fuel. Armstrong explains, "Biodiesel production starts with an oil. In the U.S. it is typically soy bean oil while in Europe it is sunflower or some other seeds.

"As these oils become converted to biodiesel, glycerin is generated as a byproduct, which, if left in the biodiesel fuel, can raise the freezing point of the fuel and clog fuel filters."

Diesel fuel tends to thicken in cold weather. If glycerin is also present, and this is more likely with biodiesel, the problem can be greatly exacerbated.

Biodiesel producers also test for minerals such as sodium, potassium, calcium and magnesium. When present, these minerals can form a soap-like substance and present problems similar to those with glycerin. "There is also concern over the presence of phosphorous in biodiesel," adds Armstrong. "The phosphorous carries over from the oil, and it can create problems inside the diesel engine.

"A number of diesel engines are now being outfitted with catalytic converters in an effort to reduce emissions. If high levels of phosphorous are present in the biodiesel, it can poison the catalyst in the catalytic converter and make it unusable."

IR spectroscopy is increasingly being used to test feedstock and other production components. "Biodiesel manufacturers typically buy oils from several suppliers, and they want to assess the quality as it comes into the production plant. IR spectroscopy is a great tool for this purpose, and it can also be used to evaluate the progress of the production process," says Armstrong.

PerkinElmer introduced its first commercial gas chromatographs more than 50 years ago. Now it offers a family of gas chromatography instrumentation, including four biodiesel gas chromatography turnkey systems, introduced in February 2007, to provide a choice of high quality analyzers for the verification of free and total glycerin in pure biodiesel (B100) to meet ASTM (Unites States) as well as the European CEN standards.

Today, PerkinElmer serves an installed base of some 130 to 150 biofuel labs worldwide. The company maintains some 1200 factory-trained and certified engineers, each with an average of 15 years of experience, who provide predictive and preventative maintenance, validation support, instrument repair and training and technical support.

Thermo Fisher Scientific provides biofuel manufacturers with the Nicolet FT-IR 380 or 6700, its most common base instruments. "The analytical process includes transmission, where light goes straight through the sample by attenuated total reflection (ATR), or by using the 'smart' arc, which requires only a small sample size for precision and accuracy. And the whole analysis can take less than 20 seconds, a fairly high throughput," says Mike Bradley, Ph.D., product specialist with Thermo Fisher Scientific.

He adds that IR spectroscopy is used on the production side of biodiesel manufacture to measure raw triglyceride content in feedstock and in post processing or blending where the goal of the analysis is to quantify the level of biodiesel.

Thermo also offers a GC-FT-IR combination instrument. "The primary advantage is that while FT-IRs are fast, the GC still has the advantage of providing greater details by taking the material apart while the FT-IR provides overall blend information," says Bradley.

Bradley explains that some instrumentation uses calibrations that require dilution of the biodiesel. "Ultimately, you need to use different calibrations for different portions of the spectrum to keep the concentration linear.

"With our instrumentation this

*continued on page 12*

*continued from page 11*

is not necessary," states Bradley. "There is no need for dilution, and one calibration framework may be used over the full range—all the way from 1% to 100% biodiesels."

Another leading analytical instrumentation developer and supplier is Brinkmann Instruments, a subsidiary of the Swiss-based Metrohm AG. Larry Tucker, business development manager with Brinkmann, says the company offers a range of instrumentation, including the Karl Fischer line, to assess moisture content in biodiesel and potentiometric titration instruments capable of assessing the quality of fatty acids in feedstock. "Titration is a quick and inexpensive way to check the quality of feedstock going into biodiesel plants," Tucker adds.

There are signs that quality service by instrument vendors is seen as a key differentiator in this sector. Tucker says, "We offer installation and training on our instruments so our customers can execute the analytical methods correctly."

Younger players are also starting to make a mark in this field. Aspectrics (Pleasanton, CA), which was founded in 2000, has developed the encoded photometric near infrared (EP-NIR) spectroscopy technology. It recently launched its multicomponent 2750 EP-NIR biofuels analyzer, which monitors methanol, water, and total glycerin in biodiesel and water content in ethanol; performs biodiesel blend determination; and analyzes ethanol/gasoline blends.

The flexible analyzer provides pass/fail information on multiple biofuel contaminants before samples are sent out for ASTM certification. A single analyzer can process multiple biodiesel and ethanol samples at 100 scans per second, generating ultra-fast, real-time results in just a few seconds.

In the next generation, more analyzers will be dedicated to specific tasks, says Bradley. "There is a change from technical staff who understood every aspect of the underlying principles of the tests to instrument users who, while still technically sound are not as familiar with the versatility of some of the systems and have to relearn them.

"Manufacturers have to ensure instruments have the capabilities of diagnosing themselves—they have to work out the calibration and the software. Plus the instruments have to be much more intelligent and, in effect, remove the burden from a lab to diagnose and fix problems.

Bradley adds that there is much progress to report on these issues. He says the footprint of the new instrumentation is dropping in size. "The instruments are becoming more intelligent by indicating to operators when they are 'sick' or what's wrong with them and when their calibration needs updating," states Bradley.

"While none of the instruments typically found at a production laboratory setting are inordinately complex," Shimadzu's Jim Mott concurs, "the proper use of this equipment is critical to the ongoing operation and profitability of the plant.

"Many of the users who will be tasked to operate this equipment may be experiencing this level of instrumentation for the first time. Therefore, the success of the laboratory is closely tied to the ongoing support of the instrumentation," says Dr. Mott.

"Because biofuel plants tend to be located where the starting materials are located, it is common to find plants in small rural areas, often far away from urban areas.

"Training programs must be considered a more than one-time effort; retraining and refresher courses are critical to user satisfaction toward the instrumentation," says Dr. Mott. ●

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## Featured New Product

*continued from front cover*

process control can now further enhance process efficiency and increase yields by analyzing ultra-high purity gases at levels that previously required far more expensive analyzers. The HALO+ is suited to challenges including fixed bulk gas continuous quality control, portable mobile analytical carts, process tool monitoring, air separation and gas cylinder quality control.

"The speed of response, accuracy, and power of Cavity Ring-Down technology are no longer the province of the elite few," said Lisa Bergson, CEO of Tiger Optics. "With the HALO and the HALO+, now many more companies can access the great performance, low cost-of-ownership, and freedom from calibration high-end users have enjoyed for over half a decade."

Designated as a transfer standard by many national laboratories, and addressing over 400 points worldwide, Tiger's analyzers are based on an absolute principle—the Beer-Lambert Law—which eliminates the need for costly and frequent calibration. Plus, they require no consumables, are robust and durable, and are easy to operate. ●

# Ensuring High Quality Biodiesel Product through Analytical Testing

*by Dave Armstrong, Hydrocarbon Processing Market Leader, PerkinElmer*

Multiple influences, including government mandates, environmental concerns, a quest for energy independence, and a desire to support the agricultural community are all encouraging communities around the world to increase their use of biofuels. Bioethanol that is blended with petroleum-produced gasoline has received widespread acceptance in many parts of the world. Biodiesel, which has been used for several years throughout Europe and Latin America, is now gaining new attention in China, Malaysia, South Africa, Canada and the United States. If consumers around the world are to make the conversion from petroleum-based diesel to biodiesel that can be used in its pure form (B100) or as a blend (such as B20), it is paramount that the producers of the biofuels provide a product that is consistently high in quality.

**At a glance**

- High levels of glycerin in biodiesel can result in deposits in storage tanks, clogged fuel filters and damaged injectors
- The testing of free and total glycerin can be achieved with full compliance with ASTM and EU standards by the use of GC
- Testing of Group I and Group II metals, as well as phosphorous, can be achieved by the use of ICP-OES
- The analysis of sulfur is achievable with ICP-OES

Industry organizations that define quality standards, including the American Society for Testing and Materials (ASTM) and the EU, have created methods that producers of biodiesel can use to certify that their biodiesel meets the quality needed to meet the need of the consumer. While these methods provide guidelines for several parameters, including flash point, viscosity, and cetane number, this discussion will focus on the tests set for the determination of free and total glycerin by gas chromatography (GC) and the determination of Group I and II metals as well as phosphorous by inductively-coupled plasma-optical emission spectroscopy (ICP-OES).

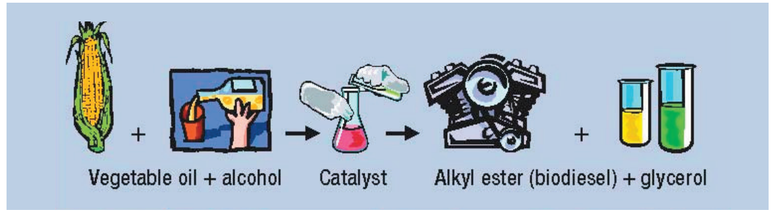


**Figure 1.** *Simplified transesterfication of vegetable oil into biodiesel (alkyl ester).*

## Free and Total Glycerine

Biodiesel is manufactured from a variety of naturally occurring fats or oils. The process, which is called transesterification, is shown in Figure 1 and produces the bi-product glycerin. High levels of glycerin in biodiesel can result in deposits in the bottom of storage tanks, clogging of engine fuel filters, and the damage of injectors in the diesel engine. To meet the ASTM standard D 6751 or EN 14105 for total glycerin, biodiesel must contain no more than 0.020 weight percent of free glycerin and no more than 0.240 weight percent of total glycerin (the sum of free and bound glycerin).

A gas chromatograph equipped with a flame ionization detector (FID) is the technology recommended by both ASTM and EU for the analysis of free and total glycerin. To determine free and total glycerin, the sample is first derivatized with a silylating agent and then injected into an open tubular GC column packed with a 5% phenylpolydimethylsiloxane. Calibration is achieved with two internal standards (butanetriol and tricaprin) and four reference materials. Mono-, di- and triglycerides are determined by comparison with mono-olein, di-olein and tri-olein, respectively. Conversion factors are then applied to the results for mono-, di- and triglycerides to calculate the sample's bonded glycerin content. The total glycerin represents the sum of the free and bonded glycerin. The peaks associated with the free and those associated with the bound glycerin are shown in Figure 2.

## Elemental Analysis

A second important test that must be performed on biodiesel if the end product is to perform as expected is the analysis of Group I and Group II metals. The transesterification reaction is quite close to the reaction used for making common soap. If the levels of Group I (Na and K) and Group II (Ca and Mg) metals are not kept at sufficiently low levels, the reaction will indeed make an unacceptable amount of soap. This soap can cause problems as the biodiesel is being used that are similar to the problems created by high levels of glycerin. Both ASTM D 6751 and EN 14538 specify the use of ICP-OES for the analysis of these metals. This analysis requires very little sample preparation. A 1-g aliquot of the sample is diluted to a volume of 25 mL using high purity kerosene. Since ICP-OES is a relative technique, the instrument is to be calibrated using non-aqueous standards in a concentration range that will bracket the anticipated concentration of the sample. At this point, the sample is simply aspirated into the instrument and the concentration read directly.

Phosphorous is considered a carry over element that is typically found in the feedstocks used in producing biodiesel. If the level of phosphorous in the final product is not controlled, the catalytic converter of the diesel engine can be damaged. ASTM D 4951 and EN 14107 do specify the use of an ICP-OES instrument for the determination of phosphorous in biodiesel. Sulphur is also a carryover element that can create difficulties with the catalytic converter of a diesel engine and can create environmental issues including the production of acid rain. At this time, both ASTM and EU recommend technologies other than ICP-OES for the determination of sulphur. While not taking issue with that recommendation, it has been found that ICP-OES instruments can readily determine sulphur in biodiesel at the levels required by both ASTM and EU. Since many biodiesel laboratories own an ICP-OES system for the analyses described above, there can be a real savings in the investment made in the laboratory if ICP-OES could be used for the determination of sulphur.

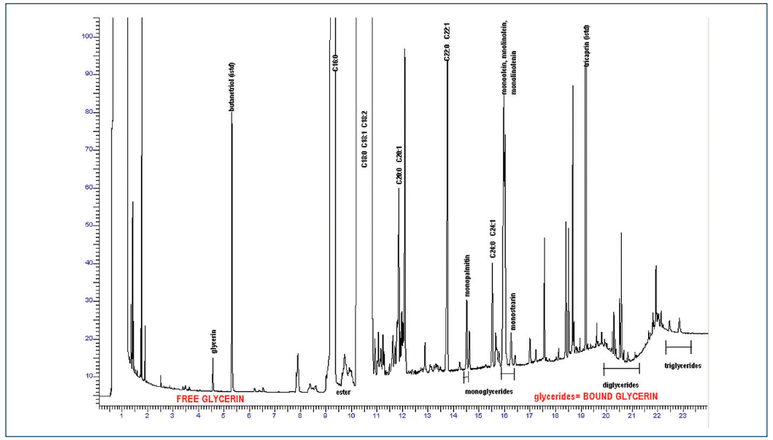


**Figure 2:** *A chromatogram of biodiesel showing the peaks used to determine free and bound glycerin.*

| | Detection Limit using ICP-OES in ppm | Levels in Biodiesel set by EN in ppm | Levels in Biodiesel set by ASTM in ppm |
|---|---|---|---|
| Sulphur | 0.01 | 10 | 15 |
| Phosphorus | 0.04 | 10 | 10 |
| Sodium | 0.0005 | **5(Sum Na + K)** | **5(Sum Na + K)** |
| Potassium | 0.001 | | |
| Calcium | 0.00005 | **5(Sum Ca + Mg)** | **5(Sum Ca + Mg)** |
| Magnesium | 0.00004 | | |

**Figure 3:** *Detection limits using ICP-OES.*

## Conclusion

As the world increases its use of biodiesel, it will remain increasingly important that the quality of biodiesel produced meets consistently high quality standards. Meeting such standards will assure acceptance by the consumer and will assure that environmental benefits possible from increased use of biodiesel are indeed achieved. The testing of free and total glycerin can be achieved with full compliance with ASTM and EU standards by the use of GC while the testing of Group I and Group II metals, as well as phosphorous, can be achieved in full compliance with ASTM and EN standards by the use of ICP-OES. The analysis of sulfur is achievable with ICP-OES; however, it is not yet in compliance with the ASTM or EU methods. Discussions are planned with both ASTM and EN to show the merits of using ICP-OES for the analysis of sulfur because it is expected that most well-equipped biodiesel laboratories will already have such a system in their laboratory for the analysis of Group I and II metals as well as phosphorous.

*For more information, contact Dave Armstrong, hydrocarbon processing market leader, PerkinElmer, at David.Armstrong@perkinelmer.com or by phone at 970-468-7656.*

### Flash Point Tester Uses Rapid Equilibrium Method for Testing Liquids

The "active cool" Setaflash tester tests the flash point of liquids and semi-solids using the rapid equilibrium method. The reliable, compact instrument is suited for use in the lab, the production line and in portable applications. When testing biofuels, the 'flash,no-flash' test method, combined with an automatic flash detector, gives a more reliable result than other flash point test methods. The device features a simple-to-use
display panel, precise temperature control, and automatic flash detection with results being displayed in either degrees C or F. Active cool is the first small scale flash point tester to provide a rapid electronic cool down facility without the need for external services. Integral electronic peltier coolers allow testing at low temperatures and rapidly reduce the test cup temperature after a test is completed. **Stanhope-Seta** www.stanhope-seta.co.uk, +44 1932 564 391

### Screeners Offer Sharp Separations

Proprietary separation equipment makes an important contribution at two critical points in the ethanol production process. When grain first enters the production process, it often contains foreign material such as rocks and other debris that must be removed. Grain Cleaners, based on the size of
the particles, separates those particles from the grain. Strategically placing these cleaners at the beginning of the process contributes to a higher quality product and reduces the maintenance costs and equipment failure that debris causes. Proprietary Screeners not only remove off-size contaminants but also offer high throughput and reliability. The rugged and durable machines are built with heavy-duty features, such as ceramic lining. **Rotex, Inc.** www.rotex.com, 800-453-2321

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# Conducting Glycerin Analysis with a Turnkey Gas Chromatography System

By David Armstrong and Tim Ruppel

Shortly after Minnesota mandated that all diesel fuel sold in the state contain 2 percent biodiesel blended with petroleum diesel, the weather turned cold and truckers and bus drivers began complaining that the biodiesel blend was clogging fuel filters. This resulted in the mandate being lifted for a total of 51 days. The Agricultural Utilization Research Institute (AURI) researched the problem and found that while biodiesel was not the only contributor to the clogging problem, it was indeed one of the contributors. Unfortunately for proponents of biodiesel, it appeared that it was the 2 percent biodiesel blended into the fuel mixture that received most of the blame for the problem.

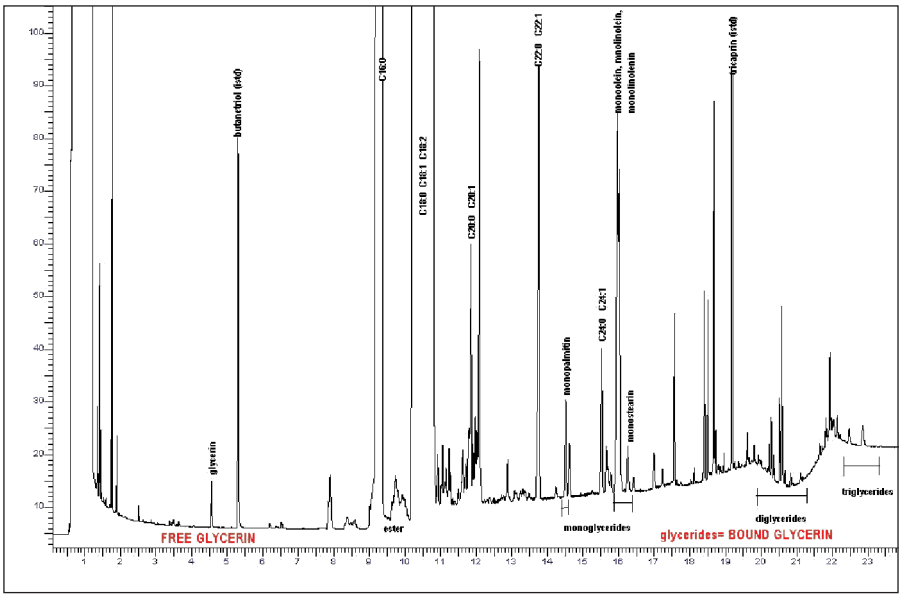


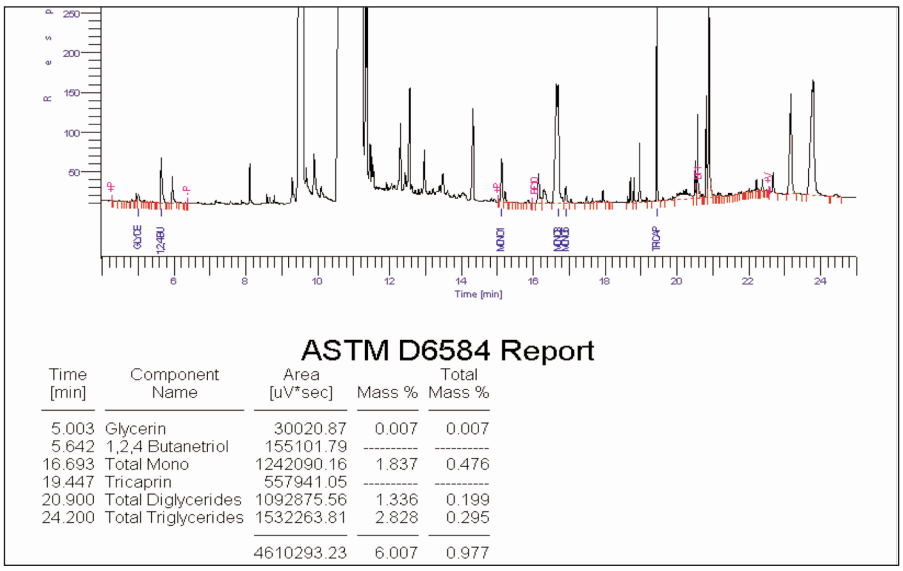

Figure 1. A chromatogram of B100 shows the peaks for free and bound glycerin.

This example and others similar to it bring to light the importance of measuring free and total glycerin in biodiesel that is to be used as a motor fuel. ASTM D 6584 and EN 14105 methods both prescribe gas chromatography (GC) as the method for this analysis. For someone who has spent years working with GC, configuring a system to perform such an analy-

sis may not seem like a daunting task. For novices at this technique, the array of letters such as PPC (programmable pressure control), POC (programmable on column) and FID (flame ionization detector) can be more than just mildly bewildering. Some of the latest technologies feature out-of-the-box preconfigured systems for performing a specific analysis like free and total glycerin in biodiesel. These systems provide a supply of consumables to analyze samples and often special training courses are available for the inexperienced chromatographer.

Having a GC system come preconfigured is most helpful, but let's not forget that standards and samples also must be prepared for introduction into the GC. Fortunately, this operation is quite straightforward and can be performed by an operator with basic laboratory skills.

For preparation of the standards, it is recommended to use a biodiesel calibration standards kit that contains reference solutions that meet the specifications called for in ASTM D 6584 and EN 14105. A set of calibration standards containing external and internal standards can be created using the kit, a pipette and the solvent heptane. The external samples will be used to build a calibration curve on the GC and the internal standards will be added to the sample itself. All external standards can be made up in autosampler vials. Sample preparation is stated in ASTM D 6584 and requires only a few steps. In a 10 milliliter (ml) septa vial, add 100 milligrams of biodiesel, 100 microliters (µl) of the internal standard, 100 µl of the derivitizing solution, shake and allow to react for 20 minutes. Dilute with eight ml of heptane and shake to mix. This solution is now ready for analysis and can be transferred to an autosampler vial. The five external standards and the samples to be analyzed can be loaded into the autosampler tray and the analysis begun.




ASTM D6584 Report

| Time [min] | Component Name | Area [uV*sec] | Mass % | Total Mass % |
|---|---|---|---|---|
| 5.003 | Glycerin | 30020.87 | 0.007 | 0.007 |
| 5.642 | 1,2,4 Butanetriol | 155101.79 | ---------- | ---------- |
| 16.693 | Total Mono | 1242090.16 | 1.837 | 0.476 |
| 19.447 | Tricaprin | 557941.05 | ---------- | ---------- |
| 20.900 | Total Diglycerides | 1092875.56 | 1.336 | 0.199 |
| 24.200 | Total Triglycerides | 1532263.81 | 2.828 | 0.295 |
| | | 4610293.23 | 6.007 | 0.977 |

Figure 2. A report generated from PerkinElmer's trademarked TotalChrom software shows the values for free glycerin (glycerin) and bound glycerin (total mono, total diglycerides and total triglycerides).

The biodiesel sample will provide a chromatogram that will appear much like the one shown in Figure 1. Peak identification algorithms built into the system's software show that the free glycerin is a single peak that elutes in about 4.5 minutes. The bound glycerides elute later and appear as three separate families of peaks. It is a good idea to compare the chromatogram from your sample to that generated from the external standards to be sure that the peaks of interest are eluding at the proper time.

At this point the software provided with the turnkey biodiesel GC system can become very helpful to identify the peak or peaks for each of these values and then calculate the concentration of each component based upon the intensity of the peak.

Free glycerin is quite easy to calculate since the GC is actually measuring glycerin itself, and the value determined by the GC can be directly reported as



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Laboratories interested in archiving their analyses or generating certificates of analysis will find that there are several new laboratory information management system products that serve this purpose very well. These biofuels specific data handling packages can collect data from all instruments in the laboratory either directly or indirectly and can use those data to provide high quality reports.

| Property | ASTM Method | Units | ASTM D-6751-06$^{\varepsilon 1}$ Standards | SoyGold Standard Ranges |
|---|---|---|---|---|
| Flash Point | D93 | Degrees °C | 130 min. | 162-174 |
| Water & Sediment | D2709 | % vol. | 0.050 max. | 0.005-0.04 |
| Kinematic Viscosity, 40 °C | D445 | mm$^2$/sec. | 1.9 – 6.0 | 4.062-4.141 |
| Sulfated Ash | D874 | % mass | 0.020 max. | 0.003-0.010 |
| Total Sulfur<br>S15 Grade<br>S 500 Grade | D5453 | % mass<br>PPM<br>PPM | 0.05 max.<br>15 max.<br>500 max. | 0.0002-0.0005<br>2-5<br>2-5 |
| Copper Strip Corrosion | D130 | | No. 3 max. | 1A |
| Cetane | D613 | | 47 min. | 48.4-49.7 |
| Cloud Point | D2500 | Degrees °C<br>Degrees °F | Report<br>Report | 0.0-2.2<br>32.0-35.6 |
| Carbon Residue 100% sample | D4530 | % mass | 0.050 max. | 0.008-0.010 |
| Acid Number | D664 | mg KOH/gm | 0.50 max. | 0.08-0.20 |
| Free Glycerine | D6584 | % mass | 0.020 max. | 0.001-0.01 |
| Total Glycerine | D6584 | % mass | 0.240 max. | 0.050-0.15 |
| Phosphorus Content | D4951 | % mass | 0.001 max. | 0.0002-0.0005 |
| Distillation Temperature, Atmospheric Equivalent Temperature, 90% Recovered | D1160 | Degrees °C<br>Degrees ° F | 360 max.<br>680 max. | 350<br>662 |
| Sodium and Potassium, Combined | UOP391 | ppm | 5ppm max | <2 |

Figure 3. A certificate of analysis of a biodiesel sample generated from PerkinElmer's trademarked Labworks Green

the free glycerin. The peaks used for bound glycerin determinations are actually peaks for various glycerides (monoglyceride, diglyceride and triglyceride), which are glycerin with additional hydrocarbon chains attached. ASTM D 6584 provides the factors needed to convert the values for glycerides in the accepted values for glycerin. The software contains these factors and automatically performs the calculation. The software then takes one more step as it adds the free glycerin value to those values calculated for the bound glycerin and determines a total glycerin value. Figure 2 (found on page 96) is an example of a report of the values for free and bound glycerin.

Laboratories interested in archiving their analyses or generating certificates of analysis will find that there are several new laboratory information management system products that serve this purpose very well. These biofuels-specific data handling packages can collect data from all instruments in the laboratory—either directly or indirectly—and can use those data to provide high-quality reports. As can be seen from the example of a report shown in Figure 3 (found on pages 98 & 99), complete data including the property, ASTM method (EN methods can also be entered), units, limit set by the method, and the standard range of the method can all be built into a report. The appropriate data from your laboratory can then be added alongside this information so that the reviewer

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| Property | ASTM Method | Units | Non-ASTM Standards | SoyGold Standard Ranges |
|---|---|---|---|---|
| Specific Gravity<br>Density@60°F | D4052 | Sp. Gr.<br>Pounds/Gallon<br>API°<br>Lbs/ Cu. Ft | Report | 0.8843<br>7.3695<br>28.56<br>55.1578 |
| Soap | | PPM | Report | ND |
| Rancimat@110 ° C | EN14112 | Hours | 3.0 | 3.0-6.0 |
| NACE Corrosion@72°F | TM0172-2001 | | B+ min. | A-B++ |
| HAZE RATING @60° F | D4176 | | 2 | 2 |
| Voluntary Filtration Method | D6217 Modified (B) | Seconds | 360 max. | 20-200 |

| | | | | |
|---|---|---|---|---|
| METALS/OTHER* | ICP | | | |
| Ca + Mg | | PPM | 5 | <0.50 |
| Cu-Copper | | PPM | Report | <0.10 |
| Na-Sodium | | PPM | Report | <1.00 |
| Ca-Calcium | | PPM | Report | <0.15 |
| Mg Magnesium | | PPM | Report | <0.15 |
| P-Phosphorous | | PPM | Report | <1.60 |
| Fe-Iron | | PPM | Report | <0.20 |
| Ni-Nickel | | PPM | Report | <0.30 |
| K-Potassium | | PPM | Report | <1.00 |

Analyzing biodiesel for free and total glycerin in compliance with ASTM 6584 and EN 14105 methods is not a trivial undertaking.

will know at a glance how the sample results compare to the criteria set by the method.

## Conclusion

Analyzing biodiesel for free and total glycerin in compliance with ASTM 6584 and EN 14105 methods is not a trivial undertaking. This task can be made much easier through the use of a turnkey GC biodiesel system that is preconfigured with all of the hardware and software needed to perform this analysis almost immediately upon installation. Ready-made standards can be commercially obtained which further reduces the complexity of the analysis. Once the analysis is performed, data from the GC, as well as data from other tests instruments, can be entered into the data handling software for data reporting and archiving.

For more information on preconfigured turnkey systems and laboratory information management system solutions tailored for the biofuels industry, visit www.perkinelmer.com/biofuels. ■

Dave Armstrong has global leadership responsibilities for the strategic marketing, planning and execution for the Hydrocarbon Processing, Biofuels and Semiconductor businesses for PerkinElmer Life and Analytical Sciences. Reach him at david.armstrong@perkinelmer.com. Tim Ruppel is the GC application specialist for PerkimElmer Life and Analytical Sciences.

# Biodiesel Concentration Measurements Using Spectrum OilExpress

**David Armstrong - PerkinElmer (USA), Sharon Williams - PerkinElmer (UK), Dave Wooten - Wooten Consulting**
PerkinElmer 710 Bridgeport Ave. Shelton, CT 06484 Phone: 1-203-925-4602 Fax: 1-203-925-4654

## Introduction

Reducing our dependence on fossil fuels and our reliance on oil and petroleum supplies are worldwide issues. Many see increasing the use of biodiesel fuel as a key initiative to meet these global needs. However, the move to include proportions of Biodiesel in everyday fuel has created a host of unresolved issues for both engine manufacturers and diesel consumers. Uppermost among these are questions concerning the concentration of the biofuel (Fatty Acid, Methyl Ester – FAME) and its quality. This application note describes how infrared transmission measurements can be used to address the concentration measurements.

Biodiesel fuels are often blended compositions of diesel fuel and esterified soy-bean oils, rapeseed oils or other potential vegetable oils, as well as fats. The physical and combustion properties of these biofuels have allowed them to achieve similar performance to diesel fuel. However, there are several characteristics (including cetane number, oxidation stability, and corrosion potential) that are of concern. These differences, especially the cetane reduction, require that adequate control of the biofuel concentration be implemented.

In addition, there are now tax incentives available in some parts of the world for the use of biodiesel. For example: in the USA this tax credit is presently in the form of a $0.01 per FAME-% per gallon of fuel used. Therefore, the difference between 19% or 20% FAME in diesel fuel can result in a considerable tax value. A recent investigation of commercially available biofuel blends identified that 18 out of 50 splash-blended samples were not the specified 20% FAME value[1]. It can be seen that there are financial justifications for an accurate biofuel concentration determination and characterization.

This work was performed using the Spectrum OilExpress system which consists of four elements:

- The PerkinElmer Spectrum™ 100 FT-IR spectrometer with high sensitivity, sampling speed and stability.
- A sealed transmission cell with zinc selenide (ZnSe) windows with a 100$\mu$m pathlength.
- The Molecular Spectroscopy Liquid Autosampler which provides unattended operation and rapid sample throughput of up to 50 samples per hour. The system is fitted with syringe pumps and is designed to handle samples with a wide range of viscosities, ensuring virtually no sample carryover (<0.1%).
- The PerkinElmer infrared quantitative software suite which allows analysis by various methodologies. These include Beer's law concentration calculations using Peak Height measurements and full Principal Component Regression (PCR) chemometric analysis.

The primary advantage of this system is the ability to automate the procedure from sample aspiration through report generation, including cleaning between samples. Secondly, the infrared transmission spectra carry the most information-rich data available, enabling more robust methods to be calculated.

## AFNOR Method using Beer's Law

One of the few defined methods for measuring the concentration of FAME is AFNOR NF EN 14078 (July 2004) – "Liquid petroleum products - Determination of fatty acid methyl esters (FAME) in middle distillates - Infrared spectroscopy method".[2]

The principle of the AFNOR method is the application of a simple quantitative model of FAME content using the 1745cm$^{-1}$ carbonyl absorbance. When using the AFNOR methods, samples are diluted in cyclohexane to a final analysis concentration of 0 – 1.14% FAME. This produces a carbonyl peak intensity range between 0.1 – 1.1 Abs, using a 0.5 mm cell pathlength. The peak height of the carbonyl band at or near 1745 cm$^{-1}$ is measured to a baseline drawn between 1820 and 1670 cm$^{-1}$. This peak height is used with a Beer's Law plot (absorbance versus concentration) to develop the calibration curve used for calculating the unknown concentrations.

While it is possible to achieve good concentration measurement, the disadvantages of this method are the need for sample dilution and the inability of the simple methodology to cope with variances in the source of the biofuel. An improved solution utilizes the more common 100$\mu$m flow-cell, avoiding the sample dilution errors. With the potential for increasing variance in feedstocks used to produce the FAME (namely: soybean, rapeseed or yellow-grease), peak area is proposed as a preferred calculation technique.

## Peak Area Method

The modifications of this method that were employed in this study included:

- Cell pathlength – 0.1 mm
- Peak area calculation – range: 1820 – 1670 cm$^{-1}$ with baseline set at the same range
- No Dilution – samples were not diluted to allow for the determination of the usable range

For a concentration method to be valid, the peak maximum cannot exceed the absorbance range of the spectrometer. Figures 1 and 2 demonstrate that the Beer's Law curve for this spectral region is limited to approximately 18% FAME.

In this study we took a baseline as defined in the AFNOR method at 1820 - 1670 cm$^{-1}$ and a peak area in the same range. The sample concentration range for this method was B0 to B16 (0% to 16% FAME). The method produced a linear graph with a correlation coefficient of 0.9988. Calculating the concentration of the standards by the method yielded a Pearson's correlation of 0.9990 and a standard error of prediction (SEP) of better than 0.30%. These results indicate an acceptable method for the quantitation of FAME up to B16.

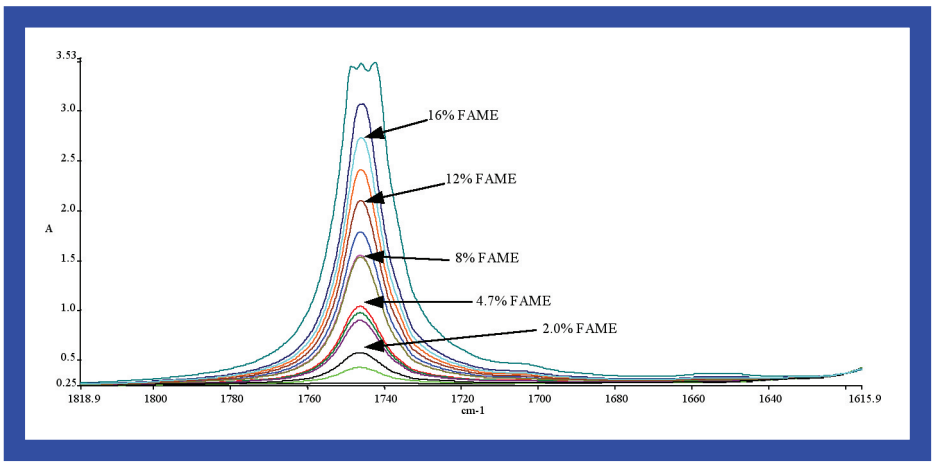


*Figure 1: FTIR Spectra of varying FAME concentrations in diesel fuel.*

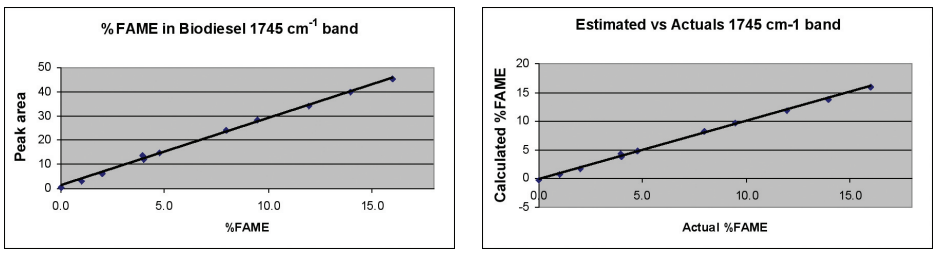


*Figure 2: Beer's Law Calibration Method for 1745 cm-1 Peak*

Further analysis of the FT-IR spectra shows additional spectral regions attributed to the FAME chemistry; for example 1300–1130 cm$^{-1}$ (see Figure 3). The peak maximum for this spectral region does not exceed the system absorbance limit even at 49% FAME. The associated Beer's Law method uses the peak area between 1300 and 1130 cm$^{-1}$. Figure 4 shows the capability of this method for an extended sample concentration range from B0 to B50. The method produced a linear correlation with a correlation coefficient of 0.9997 and a standard error of prediction (SEP) of 0.38%. This is a capable method for the determination of a wider range of FAME concentrations.

## Principal Component Regression Method

The peak area model is able to yield very capable calculations of the FAME concentration using short ranges of the full IR spectrum. To fully utilize all the relevant information from the whole spectrum, we moved to a chemometric analysis. In this case we used Principal Component Regression (PCR) to provide a more robust concentration assay. Samples with varying FAME

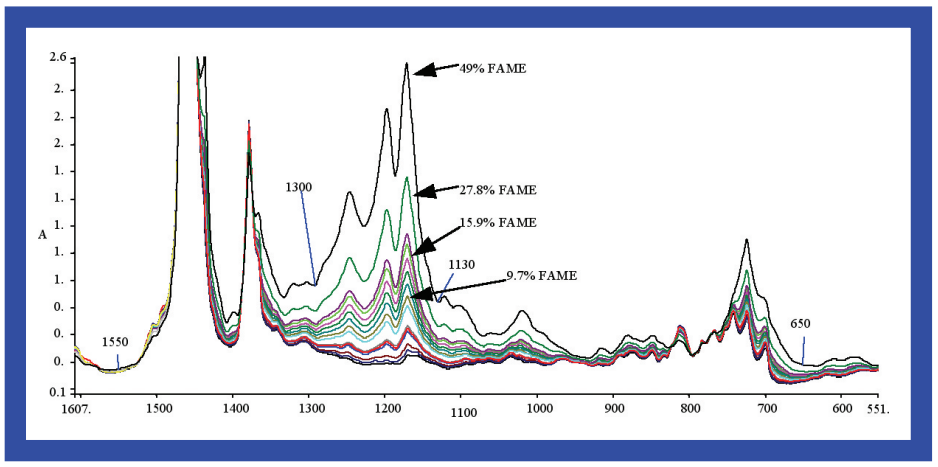


*Figure 3: Fingerprint region of FAME/diesel samples.*

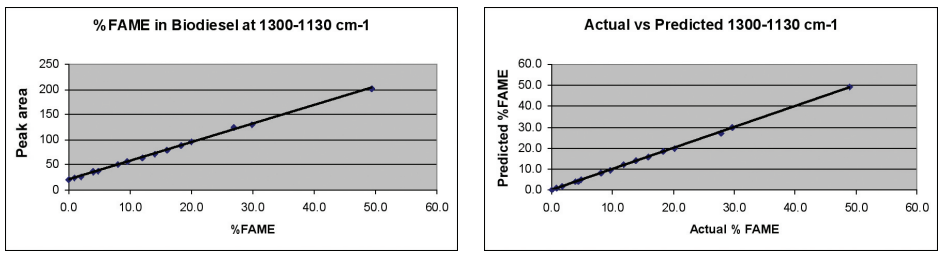


*Figure 4: Beer's Law Calibration Method for 1300 – 1130 cm-1.*

concentrations between 0 and 20% were used in the calibration of the PCR model. The model employed as much of the entire spectrum as available. The quantitative prediction utilized only one principal component (the Regression Spectrum for the method). This spectrum (Figure 5) shows that the entire spectrum was used except the top of the 1745 $cm^{-1}$ FAME carbonyl peak and the C-H peaks at 2900, 1460 and 1370 $cm^{-1}$ region.

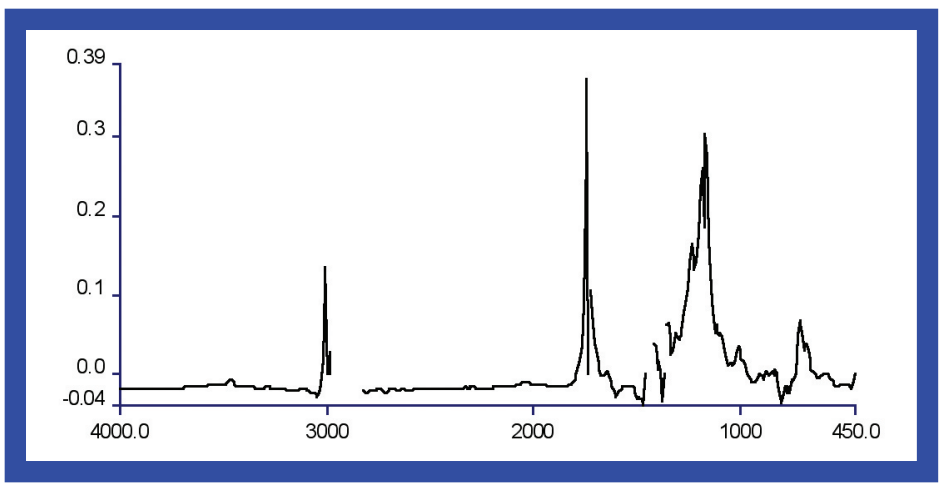


*Figure 5: FAME PCR Regression Spectrum.*

By using the entire spectral region, a more robust model can be generated. The statistics of this model showed a correlation coefficient of 0.9995, Pearson's correlation of 0.9997 and SEP of 0.17%. The actual against predicted results for this model is shown in Figure 6 also confirm a good prediction model.

This chemometric approach to the analyses is equal to or better than the Beer's Law methods. Although this modeling method for developing a calibration of the concentration of FAME in a biodiesel is more difficult to design, it is more robust over larger concentrations. Additionally it will allow extending the calibration range with additional samples to even higher concentrations.

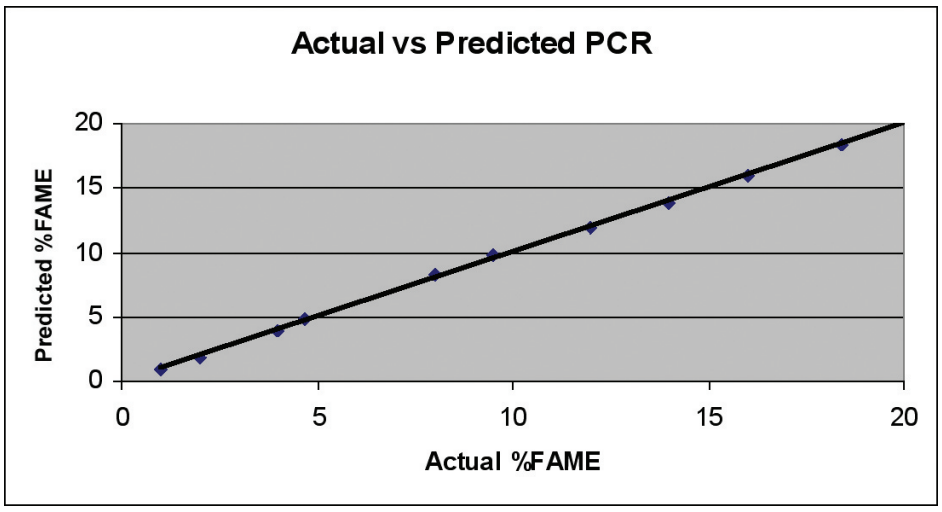


*Figure 6: PCR calibration Method.*

## Conclusions

We have shown how infrared transmission techniques can be used to address FAME concentration measurements. All the methodologies presented achieve a standard error of prediction of less than 0.4%. This compares well with the concentration measurement of FAME in a typical "splash blend" operation, where an error of 0.5% is usually acceptable. Data analysis using either Beer's Law or Principal Component Regression (PCR) is capable of meeting this requirement.

A key advantage to using the transmission cell sampling method is that it allows auto-sampling, which can ease the routine laboratory's manpower needs. The choice of either Beer's Law or chemometrics will be determined by the particular situation. The Beer's Law approach, using peak area, benefits from being a simpler approach and is recommended for situations where there are relatively few standards and low throughput of samples. The chemometrics approach has the advantage of being more robust with respect to known constituents in the blend, better handling of interferents and reducing effect from noise contributions. Overall, PCA offers higher confidence in the quantitative prediction than is found the Beer's Law methods.

## Note:

While the procedures provided in this application note may not have yet found their way into methodologies set by standard organizations or government agencies, they have been fully tested and have been demonstrated to provide quality data in numerous laboratories performing routine FAME analysis.

## References:

1. NREL Technical Report TP-540-38836, Survey Of The Quality and Stability of Biodiesel and Biodiesel Blends in the United States in 2004; R.L McCormick, T.L. Alleman, M. Ratcliff, L. Moens, and R. Lawrence October, 2005
2. AFNOR NF EN 14078 Liquid Petroleum Products - Determination of Fatty Acid Methyl Esters (FAME) in Middle Distillates - Infrared Spectroscopy Method, July 2004.
3. ASTM Proposed Standard Test Method for "Determination of Biodiesel (Fatty Acid Methyl Esters) Content in Diesel Fuel Oil Using Mid Infrared Spectroscopy"

**Acknowledgements:**
This work was performed for PerkinElmer by
David L. Wooton, PhD, of Wooton-Consulting, Beaverdam, VA, USA.

## New Analyser for In-the-field Biodiesel Screening

**Zeltex** (USA) is proud to announce its ZX-101XL portable fuel analyser is now able to screen biodiesel in the field and at the pump. For over thirteen years, state and federal governments, as well as numerous private companies have been using the Zeltex ZX-101 line of fuel analysers to accurately test octane and cetane in the field. Now the ZX-101XL can perform the same test on biodiesel fuel. With calibrations for biodiesel percentage and ethanol percentage, the ZX-101XL will prove to be the only choice for in-the-field fuel screening. Across the United States and in forty countries worldwide, Zeltex's fuel analysers have established themselves as the analyser of choice. Their analysers will provide you with lab-accurate and dependable readings within sixty seconds. Operating on "AA" batteries, the ZX-101XL can be used to test biodiesel and ethanol-blended fuels.

## New, Specifically Designed Instrument to Analyse the Oxidative Stability of Biodiesel, Diesel And Blends

For the last year **Metrohm** (Switzerland) have been developing a new instrument for analysis of diesel and biodiesel. They have improved on the existing technology of the 743 Rancimat to specifically design an instrument for the biofuels market.

During the measurement a stream of air is passed through the sample which is contained within a sealed and heated reaction vessel. These conditions accelerate the rate of oxidation of the fatty acid methyl esters in the sample, with peroxides being initially formed as the primary oxidation products. After some time the fatty acid methyl esters disintegrate completely; the second oxidation products formed include low-molecular organic acids in addition to other volatile organic compounds. These are transported in a stream of air to a second vessel containing distilled water. The conductivity in this vessel is recorded continuously. The organic acids can be detected by the increase in conductivity. The time elapsed until these secondary reaction products appear is known as the induction time or induction period.

The 873 has been modified to manage the very aggressive nature of biodiesel. Modifications include chemically resistant iso-versinic tubing and chemically resistant glass measuring vessels.

Metrohm have also proved that this principle method can be used to measure the oxidative stability of diesel and biodiesel/diesel blends. When developing the method, it was found that with volatile oils, mineral diesel and diesel blends there was a degree of evaporation on heating, resulting in wrong determination values. This was overcome by developing longer (250 mm) reaction vessels for measurements with these particular samples. These longer vessels reduce evaporation loss through the refluxer condensation principle which occurs within the longer vessels giving accurate reproducible results.

The updated software includes flexible and comfortable user administration with login functions; this can be used to define detailed access rights for groups of users and individuals. The software parameters are now also specific for biodiesel and diesel analysis.

# HPLC Analysis for the Monitoring of Fermentation Broth during Ethanol Production as a Biofuel

**Gerald Hall and Wilhad M. Reuter,** PerkinElmer, Inc.

Increased ethanol production as a biofuel is leading to a paradigm shift around the world. Renewable biological resources that can be converted to biofuels are rapidly gaining interest in the energy industry as potential alternative fuel sources. This is not just a U.S. phenomenon - it is accelerating globally. In particular, resources such as corn, sugar beats, sugar cane, grains, sorghum, molasses, and others (all renewable energy sources) are being converted into ethanol at an ever increasing scale.

The production of ethanol utilizes a fermentation process, in which yeast and enzymes convert the fermentable carbohydrates (dextrin, maltotriose, maltose, glucose) into ethanol. The resulting fermentation broth is a complex mixture, consisting of living yeast cells, nutrients, bacteria, cell debris, and other products/byproducts of the fermentation process. This broth needs to be monitored to optimize the quantity and quality of ethanol being produced. During the fermentation, it is known that the ethanol concentration is inversely proportional to the carbohydrate concentration. Therefore, the monitoring of carbohydrate levels serves as a key indicator in determining when to stop the process. In addition, other unwanted byproducts, such as lactic acid, acetic acid carbonic acid, and glycerol are also produced. To maintain productivity, these byproducts must also be monitored. During fermentation, as the composition of the broth changes, so does the chemistry. Therefore, adjustments to the fermentation broth are often required to ensure optimal ethanol yields.

This HPLC application has been designed so that, during the fermentation process, three key parameters, including eight components, can be easily monitored and quantitatively analyzed:

1) The amount of ethanol being produced
2) The amount of fermentable sugars (dextrin, maltotriose, maltose and glucose) in the fermentation broth
3) The concentration of unwanted byproducts (lactic acid, acetic acid and glycerol) produced during the fermentation process

## Experimental Conditions

The application was performed on a PerkinElmer® Series 200 HPLC System, consisting of an Isocratic Pump, Vacuum Degasser, Autosampler, Column Oven and Refractive Index Detector. TotalChrom® Chromatography Data Systems (CDS), version 6.3.1, was used as the control/data-acquisition software. The column used was a BIO-RAD Aminex® Fermentation Monitor column (150 × 7.8 mm, 5 µm).

The analytical conditions, shown on the right, were optimized to produce the shortest analysis time, while maintaining sufficient resolution between components for proper identification and quantification. Using these conditions, all components can be quantitatively analyzed in less than 10 min.

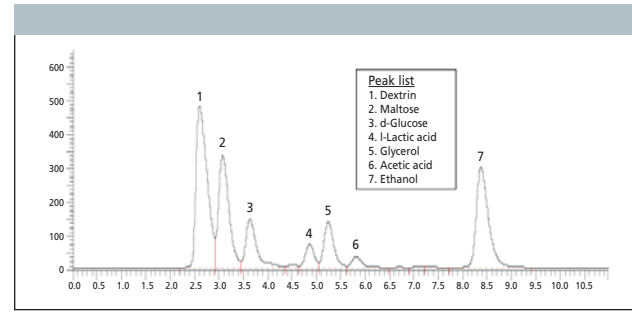


**Figure 1:** Actual 24-hour fermentation sample from ethanol production monitoring.

## Conditions

| | |
|---|---|
| Mobile Phase: | 0.001 M $H_2SO_4$ |
| Flow: | 0.8 mL/min |
| Temperature: | 60 °C |
| Detector: | Refractive index @ 40 °C |
| Injection Volume: | 10 µL |

## Results

An example of an actual 24-h fermentation broth sample that was taken during ethanol production is shown in Figure 1. From the chromatogram, it can be seen that the ethanol is well separated from all the other individually separated sugars and byproducts found in this particular sample.

## Conclusion

In conclusion, as part of the fermentation process in the production of ethanol as a biofuel, a simple ten-minute HPLC method was developed to routinely monitor ethanol, carbohydrates and byproducts. During the process, this analysis is important to help ensure that the broth chemistry is optimized to produce the maximum yield of ethanol.

## Reference

(1) U.S. Department of Agriculture - www.usda.gov

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ARTICLE

# CHROMATOGRAPHY AND MATERIALS CHARACTERIZATION

## PERKINELMER INSTRUMENTATION GOES THE DISTANCE TO ENSURE FUEL QUALITY IN INDY RACING

### PerkinElmer and fuel testing in Indy Racing

Testing and certifying fuel for purity and consistency ensures that every car competing in the 16-race 2008 IndyCar® Series schedule, including the Indianapolis 500, is on a level playing field. Fuel testing is one of more than 130 check points on a race car — from head and neck restraints to tire pressure and engine components — all to ensure that the first car to cross the finish line got there fairly.

Parked in the garage area of each IndyCar Series track is a sophisticated fuel analysis laboratory. Inside, PerkinElmer scientists analyze and report their findings of fuel composition to certify it is race-ready. PerkinElmer has been testing and certifying racing fuel since the inception of the IndyCar Series in 1996.

### PerkinElmer helps Indy Racing League go green

IndyCar Series regulations stipulate that the fuel must be 100 percent fuel-grade ethanol, which is a blend of 98 percent ethanol with 2 percent 98 octane unleaded gasoline. The IndyCar Series, guided by PerkinElmer experts, migrated to fuel-grade ethanol in 2007 to achieve more efficient engine performance and contribute to the lessening of dependence on oil-based fuel.

Brett Boyer, Senior Service Engineer, PerkinElmer Life and Analytical Sciences, runs the onsite testing lab. PerkinElmer is the official instrument supplier and fuel certification partner for the Indianapolis 500 and the IndyCar Series. Boyer and his PerkinElmer team test and certify fuel purity and consistency from the source of the fuel and through the supply chain to onsite on race day at IndyCar Series races in the United States.


### From methanol to ethanol

For many years, 100 percent methanol was used in the high-performance race car engines. In 2006, the IndyCar Series introduced a blend of 90 percent methanol and 10 percent ethanol as a transition fuel before full incorporation of ethanol. The IndyCar Series' conversion to the renewable fuel was introduced by the late Paul Dana, a driver who was killed in 2006 during a practice session, and was supported by the Ethanol Promotion and Information Council (EPIC), a non-profit alliance of ethanol industry leaders.

### The use of the Clarus GC for fuel analysis

PerkinElmer tests use its Clarus® chromatography (GC) controlled by a PerkinElmer TotalChrom® chromatography data system for collecting, processing and reporting data. The Clarus GC separates the fuel to identify additives that may give one car a competitive advantage over others. The testing takes approximately 5 minutes and can detect impurities down to concentrations of 0.10 percent.

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Kevin Blanch, Technical Director for the IndyCar Series, relies on PerkinElmer for testing the fuel in each car a minimum of three times over the course of a racing weekend. The analysis yelded positive results upon occasion.

To determine the ideal fuel blend, PerkinElmer and IndyCar Series engineers tested a range of options using a dynamometer to gauge the BTUs and RPMs. The transition required minor calibration changes only since the IndyCar Series cars were already running methanol, another alcohol-based fuel. To further protect engine integrity, a Honda engineer is assigned to and stationed with every car to troubleshoot engine issues. Series officials also decided to reduce the size of the fuel cell from 30 gallons to 22 gallons, because ethanol is more fuel efficient than methanol.


## The use of the Spectrum™ 100 in engine lubricant analysis

The composition of engine oil also can impact performance. PerkinElmer analyzes the race cars' engine oil lubricants for contaminants and conformance to IndyCar Series requirements. The PerkinElmer Spectrum™ 100 infrared spectrometer is used for both the IndyCar Series and Indy Pro Series and provides instantaneous results to ensure contaminant-free performance. With the conversion to ethanol, the IndyCar Series offers fans and drivers alike all of the fast-paced excitement they've come to enjoy — and the added benefit of a safe, renewable energy source that ensures that the IndyCar Series is on track for a safe, clean and environmentally sound future.

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QUALITY


# INDY'S SUPER FUEL

Since 2006, Indy Racing League competitors have been using ethanol to fuel their hopes for a checkered flag. Making sure that quality is never sacrificed in the process is the job of PerkinElmer Inc. EPM goes to the Milwaukee Mile to experience the power of Indy racing and to see firsthand what goes into testing the fuel for this high-octane environment.

Story and Photos By Craig A. Johnson

QUALITY

Screaming around the track at more than 160 miles per hour, an Indy racing car skims a razor's edge between success and chaos. For the 50,000 fans in the grandstands, ear protection is not an option, but a necessity. At the Milwaukee Mile, a "small oval" by Indy standards, 27 race teams converged recently to risk their reputations for a chance at victory.

The last Saturday in May is qualifying day and crowds swarm pit road eager to catch a glimpse of their favorite driver. Some of the biggest names in racing are here. Favorites such as Marco Andretti and A.J. Foyt converse with newer stars like Danica Patrick, Tony Kanaan and Helio Castroneves, who appeared on ABC television's "Dancing with the Stars" and won.

Fans are understandably excited by the approachability of these icons. And the cars are no less famous. They are as sleek and muscular as jungle cats. Nothing on these vehicles is overlooked as every ounce of material supports the dual emphases of safety and speed.

Tolerances in Indy racing are incredibly thin, a function of the environment they inhabit. In the five closest Indy Racing League races, the *combined* margin of victory was .0275 seconds. That's about one-fifth of the time it takes to blink once. Each piston travels more than a mile up and down in its cylinder each minute. The wind force put on the car at top speed would allow the cars to race upside down. It is to this dance that ethanol was invited.

An Indy Racing League official fills a tank with racing-grade ethanol.

## A Brief History of Racing Fuel

In 2005, the IRL used 100 percent methanol in their tanks. The fuel worked well and was more efficient than straight gasoline, but IRL officials believed there was room for improvement. In 2006, they moved to a 10 percent blend of ethanol and methanol, which was then changed to 100 percent ethanol for the 2007 season.

The desire to use only ethanol aside, 100 percent ethanol is nearly impossible to obtain, and is illegal to keep without paying hefty taxes that date back to prohibition. The actual fuel used by the IRL is a blend of about 98 percent ethanol and 2 percent denaturant. The exact composition includes a small amount of high-octane racing fuel. The fuel for today's Indy cars has an octane rating of 113, compared with methanol's 107. This means a significant reduction in pre-ignition, or knocking and pinging.

In addition, many of the people who handle the fuel, say it smells like tequila. This quality may seem insignificant, but smell is actually one of ethanol's benefits when compared with methanol. Pit crews noticed the switch to ethanol immediately, reporting fewer coughing fits and less eye irritation before any other modifications were made to accommodate it.

According to Dennis Vervynckt, manager

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Ryan Hunter-Reay's crew rolls the ethanol car in to be tested.

of hospitality services and an IRL official, in 2006, the first year the IRL began using ethanol, teams saw a reduction in efficiency of about 10 percent. "That's because we put straight ethanol into the tanks," he says. "The teams initially didn't make any—or very many—adjustments to their engines. After a few months, they kept tweaking and by the end of the season they were back up to 100 percent efficiency."

Vervynckt points out that there was a minor loss of power. "When they first started using ethanol, teams saw a slight drop in horsepower," he says. "As the teams got used to the fuel, they were able to get more horsepower from ethanol once they again reached maximum efficiency."

The switch to ethanol also allowed the racers to carry less fuel and make fewer pit stops, thereby increasing efficiency even more. "When the cars ran on methanol tanks had to hold 30 gallons to accommodate the fuel requirements of the cars," Vervynckt says. "After getting the engines to perform at their full potential, there was a significant gain in mileage. Indy cars now have 22-gallon tanks. When a driver pulls in to change out his tires, he can fill up. Teams were able to match their tire and fuel stops exactly, instead of stopping for tires only, or fuel only."

That efficiency translates into much less fuel being used by the sport. Lifeline Foods LLC, which supplies fuel for the IRL, will produce 120,000 gallons of ethanol for the 2008 season. And though this may seem like a lot, it's 20,000 gallons less than was used the previous seasons.

The switch from methanol-based fuel to ethanol has amazed almost everyone in the league. Drivers, the direct users of the fuel, voice their approval in glowing terms. "[Ethanol] is an American fuel that is good for the country and the environment," says Ryan Hunter-Reay, of Rahal Letterman Racing and driver of the ethanol car. Standing beside his crew as they make last-minute adjustments to the car, he is more than happy to discuss the benefits of the IRL's switch to ethanol. "It's been great for Indy racing—the fuel economy, emissions—there's nothing we don't like. We love it."

## PerkinElmer's Testing Procedure

During Saturday's qualifying round cars must make a date with IRL race official Kevin "Rocket" Blanch. All day teams roll their cars from their setup areas in the infield to the testing center for inspection.

The cars come in order—no exceptions. As teams push them toward the testing area, Blanch makes sure they know who's in charge. "Go! Go! Go!" he yells to a team that's taking a little too long to get their front wing set before rolling underneath the white awning that's popping in the strong wind off Lake Michigan.

Outside the tent, two men in red shirts dash from car to car taking small samples of fuel. Gerry Kennedy, a senior customer support engineer for PerkinElmer Inc., handles a syringe with a long tube. After taking a bit of fuel, he turns to PerkinElmer field service engineer Saleh Abdeljalil, who stands ready with a small sample jar. Abdeljalil makes a note of the sample number and then places it, in order, in a piece of wood that holds the sample jars. As Abdeljalil finishes, Kennedy is already headed for the next car.

Fuel testing takes place before and after qualifying. "We test the fuel from the main tanker truck and then from all the cars," Kennedy says. "After the qualifying round, the top five teams will have to come back and we'll do a follow-up test."

Of course, stiff penalties await any team that might attempt to cheat, but the idea might

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Blanch, center, keeps the testing area running smoothly.

be more apocryphal than realistic. "There's not much they can put in the tank that would give them an advantage," Abdeljalil says. Still, the fuel needs to be checked, and if a sample deviates too much from the mean, further testing will be done.

PerkinElmer partnered with the IRL in 1996 to begin testing fuel for the league using gas chromatography. Before then IRL fuel was tested using ultraviolet-visible spectroscopy (UV-VIS). Analyzing the absorption of light passing through the sample, UV-VIS is a fairly easy test to perform. The test is accurate to a point, but gas chromatography is preferred for its ability to identify the actual composition of the elements and particles present in the sample. A UV-VIS test can tell if fuel has impurities in it, but it can't necessarily tell what all of those impurities are. Conversely, a gas chromatograph can tell exactly what impurities are in the fuel based on their chemical signature.

After the cars are through the checkpoint and given the green light to qualify, they are rolled to pit road for any last-minute preparations before the drivers arrive. The samples taken by Kennedy and Abdeljalil are brought inside the IRL station and tested using PerkinElmer's gas chromatograph (GC). As the qualifying round commences, most Indy officials begin to turn their attention to the television screens. Everyone is a race fan whether they are directly involved with the IRL or the racers, or not.

In the meantime, PerkinElmer's scientists still have a job to do making sure each of the 27 samples is accounted for and will be checked accurately. Abdeljalil and Kennedy are oblivious to the cars still audible zooming around the track.

"Samples go into the GC unit and are heated," Kennedy says. "They start at about 45 degrees Celsius (113 degrees Fahrenheit), increasing by 20 degrees each minute up to about 160 degrees C (320 degrees F). Each sample takes five to seven minutes, so once we start the samples will take a few hours to process."

The next day, when all the samples have been processed, and assuming all comes out well for the teams, Kennedy and Abdeljalil will only need to test the top five finishers. "The only problem is that sometimes they forget to bring their car by so we can test it," Kennedy says. "That means we may have to track them down quick and get the sample." Because the fuel is pumped out of the cars before they travel, the sense of urgency is real. "There have been a few times I've had to stick my arm way down in the tank just to feel for any moisture whatsoever," Kennedy says. "Luckily we don't need much for the GC."

## Ethanol in Indy's Future

PerkinElmer is one of many players who make the IRL the powerhouse it is in American racing. From the technologically advanced tires that Firestone produces for the league, to the engines—all identical from the factory and leased by Honda—Indy racing brings a powerful image to anyone affiliated with the sport.

The fact that the IRL switched to ethanol should signal to observers that league officials spent a lot of time and energy making sure they were getting the best fuel available. Their ringing endorsement is an indication that they plan to continue the relationship for a long time to come. EP

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