Measurement of Biodiesel Blend Ratios Using a New Field Test Kit

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Introduction

Biodiesel (B100) is available blended with conventional diesel fuel in most every state in the union. In fact, it may be in the fuel you just bought because the FTC does not require labeling of biodiesel blends containing 5% or less of B100[1]. Once B100 is blended with petroleum diesel to create a biodiesel blend, it is impossible to tell by observation how much B100, if any, is contained in the blend. Verifying B100 content requires a laboratory analysis which, for many blenders/consumers requires an off-site lab. For blenders/consumers who do not maintain a lab or who cannot afford the equipment, one solution is to use field test kits. Until now, a chemical field test kit for biodiesel content did not exist. With the introduction of Dexsil's FAME Check this year, blenders and biodiesel consumers can verify B100 content without sending samples to a lab. This paper provides a brief overview of FAME Check and its capabilities.

Background

Biodiesel or B100 is defined by ASTM as "fuel comprised of mono-alkyl esters of long chain fatty acids derived from vegetable oils and animal fats."[2] Typically, the triglycerides are converted via a transesterification reaction with methanol and a base to produce the fatty acid methyl ester and glycerin as a byproduct.[3] As feedstocks, virgin plant oils are often preferred due to their uniformity and purity, but animal fats, waste products and byproducts from other industrial process can be used. Other common sources are tallow and yellow grease produced in rendering plants and vegetable oils used in frying foods. The annual biodiesel production in the US has exceeded 1 billion gallons since 2011 and in 2013 it was nearly 1.8 billion gallons.[4] As B100 production has grown it has become more available for blending into petroleum diesel resulting in blended fuels being available in all 50 states and Canada. As of this writing, there are incentives in place in almost every state promoting the use of alternative fuels including biodiesel. In Minnesota and Washington state there are minimum B100 content requirements in place.

The "bio" content of a biofuel blend, in this case biodiesel, can be determined after the fact using laboratory methods. The ASTM method (D-7371) for biodiesel content in blended fuels is based on FT-IR analysis and requires calibrated instrumentation and a laboratory.

A field method would fill a need for small to medium sized companies who do not have the facilities for on-site lab analysis.

Method Development

The primary chemical feature of biodiesel which would allow it to be distinguished from petroleum diesel is the presence of the ester group. The stability of this bond is what makes methyl esters suitable as fuels. It also makes them difficult to quantify in blended fuels. This difficulty manifests in laboratory methods and field methods alike. Early GC and GC-MS methods required cryo-focusing or differential flow modulation techniques[5] to separate the FAME components from the diesel fuel. The current ASTM FT-IR method requires calibration standards specific for different types of base fuel.

The approach taken in the development of the FAME Check field test kit was to convert the methyl ester component of the biodiesel into a quantifiable form. After much research and experimentation, it was determined that the most direct conversion was the best. By converting the methyl ester into the free acid and isolating the product, the fatty acid concentration can easily be determined by a simple titration.

Feedstock	Weighted Average Molecular Weight*	RPD
Canola	293	1.2
Corn	290	-0.1
Lard (Y Grease)	283	-2.4
Palm	277	-4.6
Rapeseed	312	7.7
Soy	291	0.2
Tallow	275	-5.2
Median	290	
*Calculated Using Data from Refs. 3 &6		

At room temperature, the conversion to the free acid will proceed slowly in the presence of acid or base. By using concentrated base and a catalyst, the reaction goes to completion in 5 minutes with some agitation. Once the conversion is complete, the excess catalyst and base must be removed from the sample so that the free acid can be titrated. This is accomplished by adding a solvent and a large excess of aqueous acid which protonates the acid, washes out the excess base and forces the free acid into the organic layer. This liquid/liquid extraction also removes any acidic contaminants in the fuel caused by oxidative degradation which would interfere with the test. A sample of the organic layer is then titrated to a colorimetric endpoint using a sodium hydroxide solution. At the titration endpoint, the equivalents of acid in the sample equal the equivalents of base added so the results can be calculated on a molar basis. The industry standard for biodiesel blends is to use volume percent to blend fuels. To convert the results it is necessary to know the molecular weight of the B100 and the density. As can be seen in the table above, the average or weighted molecular weight for most FAME mixtures derived from typical feed stocks are on the order of 290 varying by less than 10% from the median.[6] Using the nominal molecular weight of 290 and a nominal density of 0.875 g/cc the conversion to volume

percent can be made with only a small (less than 1%) expected error for a 10% biodiesel blend. As will be shown, this conversion holds for all of the B100 feedstocks tested and was empirically verified in the calibration of the titration burettes. By calibrating the titration burette in units of volume percent, the FAME content of the original sample can be read directly on the side of the burette. NOTE: Some feedstocks may contain a large fraction of low molecular weight free fatty acids (FFAs) primarily lauric acid (12:0)[7] resulting in a FAME mixture with an average molecular weight significantly different from 290, e.g., coconut oil with an average molecular weight of 218. Using the above calculation, this would result in an absolute error of 2.2% over estimation on a 9% blended fuel. If the source of the B100 is known, a correction can be made to the final result to yield the correct answer.

Experimental

During development, the kit was used on many types of biodiesel from a variety of sources. Some of the B100s used were made in-house, but the majority of them were obtained from commercial producers. In addition to using B100, standards were also formulated using methyl oleate. NOTE: Over the course of this research it became increasingly difficult to find commercial diesel fuel without B100 because of its widespread use and the lack of a labeling requirement for blends containing less than 5%.

The biodiesel blend standards used were made up using a volumetric flask and a glass airtight syringe. The volumetric flask was first partially filled with commercial diesel fuel (without a B100 component), a specified volume of B100 was added to the fuel using the syringe and the mixture was taken up to volume using more diesel fuel. The flask was capped and inverted and the final volume verified. All standards were transferred to a screw-topped glass vial for storage till used.

The first experiments to verify that the reaction conditions had been optimized were run using methyl oleate spiked in diesel fuel available at the retail pump. Methyl oleate was used because it is a discrete compound with a known molecular weight, the purity of which could be verified using GC and GC-MS methods. Using standards of known molar concentration the reaction efficiency and overall recovery could easily be calculated. The optimized procedure was then tested on the mixed FAME B100 samples in diesel fuel. The calibration of the titration burette was then adjusted to account for the overall recovery so that the end user could read the concentration directly in volume percent.

The finalized production kit was then used to perform a series of experiments with different operators and blended fuels derived from various B100 feedstocks.

In developing a commercial version of the test, some consideration was given to the ease of use, packaging and shipping, as well as keeping the overall cost of the test down. The production

version of the test is packaged as a single use disposable kit. Each test is packaged in a box roughly the size of a VHS cassette and contains everything necessary for one test. Kits are sold in packs of 20 and cases of 80 tests.

The components of the test are essentially a reaction tube and a titration tube, each of which are soft sided polyethylene. The reagents are pre-measured and are contained in either screw-top or break-top vials. Included in each test are preset sampling syringes and a titration burette calibrated in volume percent.

The procedure for running the FAME Check kits consists of the following steps:

    1- Introduce the sample into the reaction tube using the preset sampling syringe.

    2- React the sample with concentrated sodium hydroxide and a catalyst. (10 minutes)

    3- Extract the FFAs into the organic solvent (5 minutes) and transfer a portion to the titration tube.

    4- Titrate FFAs to a light blue endpoint shown below and read answer on side of burette.

PRE-ENDPOINT   ENDPOINT   TOO FAR
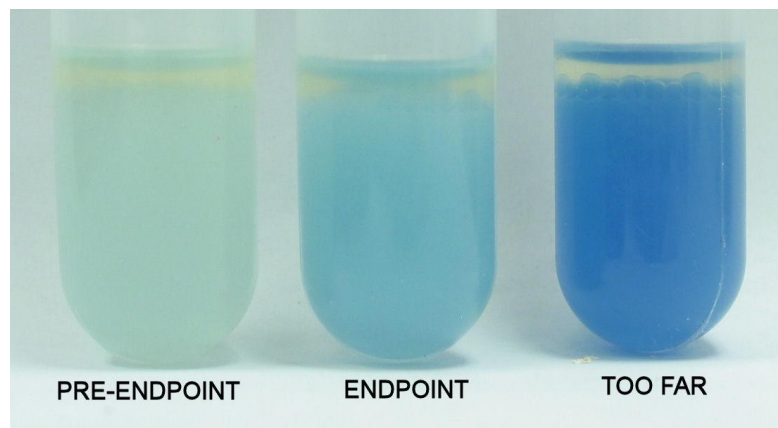
The entire test takes approximately 20 minutes to run, due to the reaction time for conversion of the esters to free acids and the separation of the organic solvent from the reaction mixture. The reaction step requires only 1 minute of shaking, so multiple tests can be run simultaneously to improve throughput. Each of the steps is described in detail in the kit instructions and the endpoint is easily identified.

Results and Discussion

During the development of the test kit one of the design criteria was the reproducibility. Since the test involves a number of steps, it was important to insure that any variation in the individual steps, e.g., conversion to FAME, extraction into the organic layer, titration of the extracted FFAs, etc., could be minimized so as not to affect the overall accuracy of the test. Conversion or extraction efficiencies could be accounted for in the final conversion equation to volume percent, but inconsistencies or variable outcomes would mean the precision of the test would suffer. The USEPA defines a test procedure for determining the lower limit for analytical method known as the MDL (Method Detection Limit). The procedure as outlined in 40 CFR[8] is a calculation of the 99 percent confidence interval (CI) for a series of seven replicates of the method on a low level sample. Using a soy based B100 to spike pump diesel fuel at 1.5 V% the MDL procedure was applied to the production test kits. The results tabulated at the left, indicate that the MDL for the method is 0.362 V%. Using the standard deviation from this replicate data to calculate the 95% CI for this data results in an interval of $\pm$ 0.106 where $CI_{0.95}=\pm t_{0.025}(s/\sqrt{n})$.[9]

Test Number	FAME Check Result (V%)
1	1.323
2	1.469
3	1.469
4	1.469
5	1.469
6	1.713
7	1.469
avg	1.483
St. Dev.	0.115
MDL	0.362

Because the presence of the FFAs in the extract changes the polarity of the solvent, it is important that at increasing levels, the FAME in biodiesel does not change the recovery of the produced FFAs. To help minimize the possibility of separation issues, the sample volume was kept small relative to the solvent volume. As a demonstration of this, a series of diesel fuel samples spiked with methyl oleate over a range of 0-9 V% were analyzed in duplicate using production kits. A statistical analysis of the data was then performed to confirm the linearity and accuracy of the kit. The diesel fuel was spiked at 2, 5 and 9 percent by volume. Along with a blank fuel sample, the results are tabulated below.

Theoretical Volume %	Measured Volume %
0	0
0	0
2	2
2	2.125
5	5.125
5	5.25
9	9.125
9	9.325

A plot of the data demonstrates the linearity of the kit response (R^2=0.996) and the slope indicates a statistically insignificant positive bias of 2.5%. NOTE: This is a relative percent which translates into a bias of only 0.225% absolute on a reading of 9%, i.e., a 9% sample would read 9.225, estimated. It should also be noted that the intercept of the regression line is not statistically different from 0 indicating that there is no systematic bias to the results. This means that diesel fuel with no bio content would reliably read 0.

FAME Check Measurement of Methyl Oleate

$y = 1.0258x + 0.0155$
$R^2 = 0.9996$
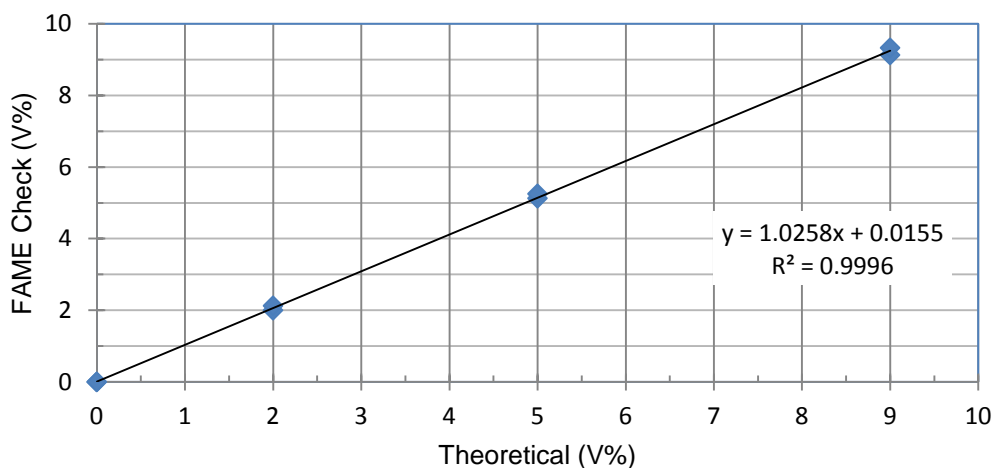
As mentioned above, the feedstock for the production of biodiesel will affect the molecular weight distribution of the FAME in the final product. This variation could be as high as 8% for rapeseed oil with its high Erucic Acid content (13c-22:1), but for most of the typical feedstocks used in the US, it should be on the order of 2-5%. When blended at 10% in a biodiesel blend, the effect would be on the order of 0.2-0.5% variation in the measured result.

Using B100 from a variety of feedstocks to spike diesel fuel samples from 0-10V%, a composite curve of kit results illustrates the desired lack of sensitivity to molecular weight variations. Based on molecular weight considerations, the largest difference would be between canola oil and lard produced FAMEs. The difference

B100 Blend Standard (V%)	Mixed Source Reading (V%)	Canola Reading (V%)	Cooking Grease Reading (V%)	Lard Reading (V%)	Corn Reading (V%)
0	0.153	0.000	0.153	0.153	0.000
0	0.006	0.153	0.153	0.006	0.104
1	0.981	0.835	1.030	0.981	1.030
1	0.981	0.738	1.030	0.981	1.030
2.5	2.444	2.444	2.493	2.444	2.444
2.5	2.444	2.493	2.590	2.688	2.444
5	5.223	4.881	5.369	5.125	4.881
5	5.125	4.881	4.881	4.759	5.125
9	9.513	9.025	8.928	9.147	8.928
9	9.025	9.025	9.123	9.396	9.025

should be about 3.6% with lard being the lower. The resulting difference on a 9% sample should be approximately 0.32%, lard should read higher than canola oil. From the table, the average lard result for the 9% sample is 9.271 and the average canola is 9.025 a difference of 0.246%. While this is clearly in the right direction, this difference is not statistically significant which is

true for all of the B100 sources. The differences in the results for the various sources of B100 are within the variation of the test and cannot be distinguished.

A plot of the above data illustrates the lack of effect feedstock has on the test results.

Multiple B100 Feedstocks
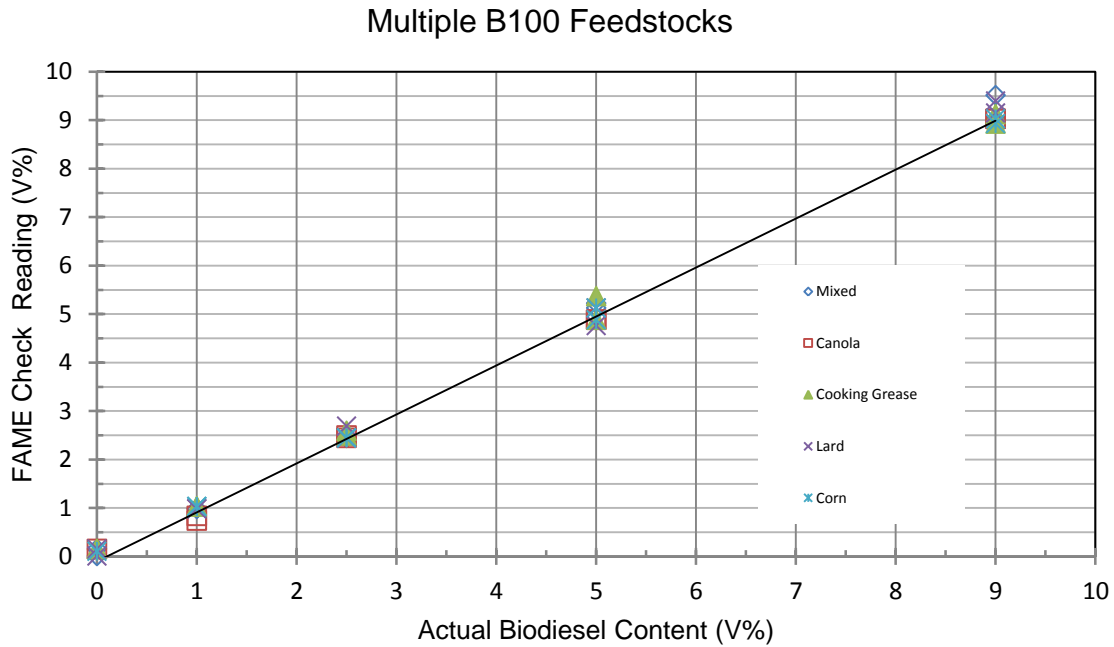
To insure that the R&D lab personnel were not hyper-trained and prone to overcompensate when running the test, outside operators were brought in to run the test using blind samples. For this series, diesel fuel samples were spiked at 2.5, 5, and 9V%, aliquoted into 1 mL sample vials and labeled with random numbers. Each operator analyzed each sample including an unidentified duplicate for each of the spike levels. The results, tabulated below, demonstrate how reproducible the kit results are. Note: All of the results are well within the 95% CI calculated in the MDL experiment as compared to the expected value.

B100 Blend Standard (V%)	Operator 1	Operator 2	Operator 3
2.50	2.10	2.25	2.13
2.50	1.85	2.00	2.13
5.00	4.75	5.00	4.50
5.00	5.00	4.75	4.88
9.00	8.75	9.00	9.00
9.00	8.85	8.75	8.88

A plot of the data illustrates the linearity and the repeatability of the data.

Multiple Operator Results
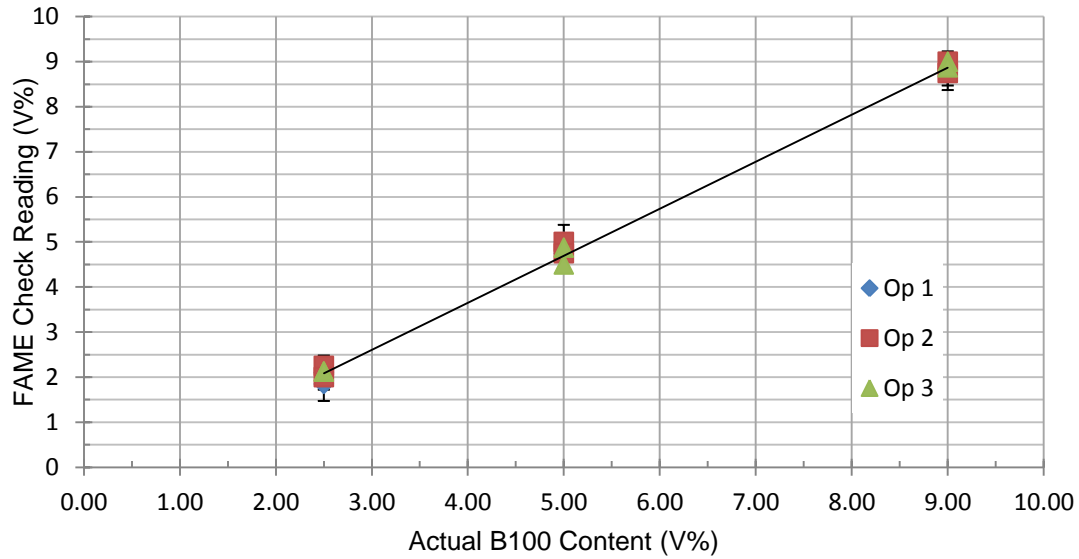
Feedstock	Vol % B100	FAME Check Raw Reading	Corrected Reading (V%)
Methyl Oleate	0	0	0
Methyl Oleate	0	0	0
Methyl Oleate	2	0.75	1.5
Methyl Oleate	2	1.125	2.25
NOCO (Mixed)	5	2.6	5.2
NOCO (Mixed)	5	2.5	5
Methyl Oleate	9	4.625	9.25
Methyl Oleate	9	4.625	9.25
NOCO (Mixed)	9	4.625	9.25
NOCO (Mixed)	9	4.625	9.25
L148 (Soy, Argentina)	12	6	12
L148 (Soy, Argentina)	12	6.1	12.2
NOCO (Mixed)	14	7.125	14.25
NOCO (Mixed)	14	6.875	13.75
Lard	17	8.5	17
Lard	17	8.375	16.75
Methyl Oleate	18	9	18
Methyl Oleate	18	9.375	18.75

In the event that there is a demand for a test with a larger range due to increased use of higher percentage biodiesel blends, a method for extending the range of the FAME Check to 20% was investigated. The higher range uses a smaller portion of the FFA containing organic layer for titration to achieve the higher range. By using exactly one half the volume for titration, the final result is simply twice the reading off the titration burette. Using mixed B100 blends from different feedstocks over the range from 0% to 19% duplicate analyses were performed on each concentration.

Based on an analysis of the elements of the test contributing to the overall variability, the limiting factor determining the minimum error possible is the resolution of

the burette. The error bars calculated from the MDL experiment are on the order of one division on the scale. There are other contributing factors such as the conversion efficiency to the FFA and the recovery into the final organic layer along with the volume of the original sample. These factors do contribute some to the overall error, but the ultimate limiting factor is the final titration. Due to the multiplier to convert the results to the final concentration, the expected error bar, in absolute terms, will be twice the error achievable for the 0-10% test.

Extended Range FAME Check Using Different Biodiesel Feedstocks

$y = 1.0121x - 0.0375$
$R^2 = 0.9981$

(Y-axis: FAME Check Reading X 2 (V%); X-axis: Theoretical (V%))
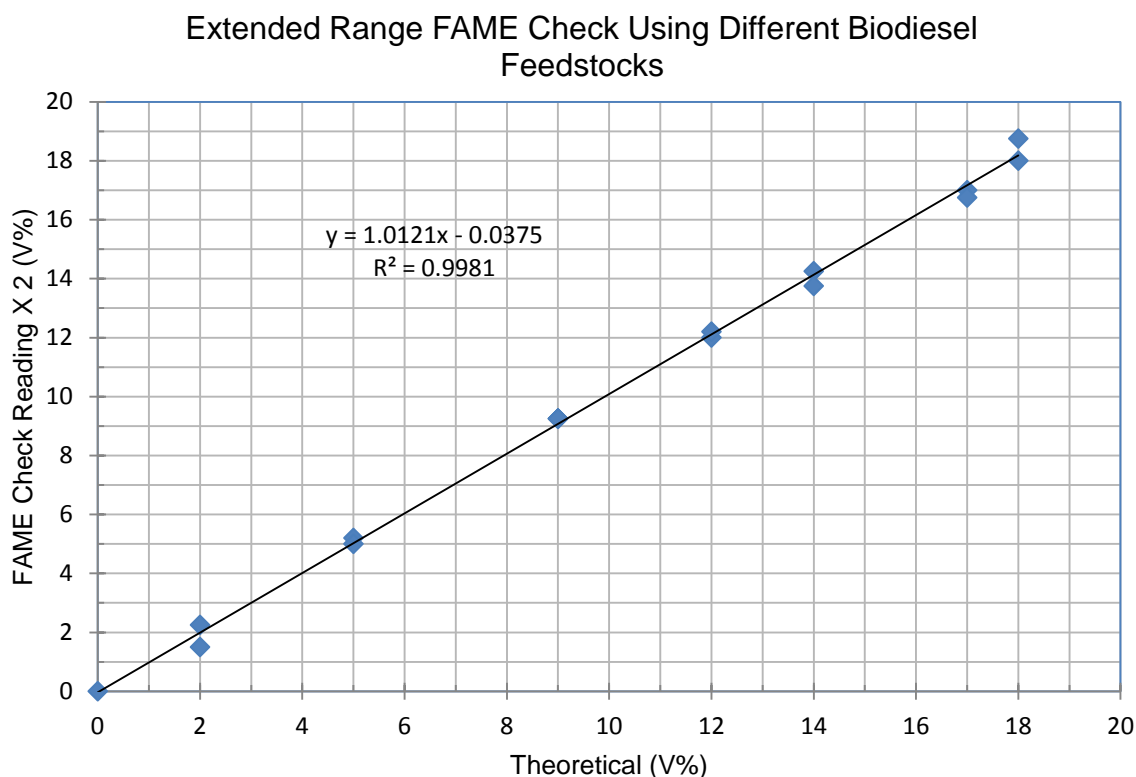
Conclusion

Over the course of the development of the FAME Check test kit, the market place for biodiesel has changed from a backyard operation with few standards and even fewer commercial vendors, to a much more standardized and regulated environment with biofuels available in every state. In the early days, much of the concern was over quality control issues with the finished product. Through the systematic adoption of standards and establishment of controlled procedures, the industry has matured into an integrated industrial production network. Biodiesel has become an established part of the biofuel landscape.

With B100 practically everywhere, it is often necessary to have a quick test available to determine if the fuel you are getting actually contains the required level of biodiesel, or conversely contains biodiesel when it shouldn't. The repeatability and accuracy of the FAME

Check test kit demonstrated here recommends it for everyday determination of blend ratios in diesel fuels. The MDL of 0.36 V% is low enough to determine if B100 has been blended with the diesel fuel in question. The precision and linearity up to 10 V% makes the kit suitable for determining exact blend ratios in the most common range for available fuels. These results were repeated on most types of feedstock B100 indicating that there should not be a noticeable regional variation in accuracy due to the source of the biodiesel.

[1] 16 CFR Ch. 1, Part 306.12 Labels, Append. A, "Summary of Labeling Requirements for Biodiesel Fuels," 1-1-12 Ed.

[2] ASTM D6751-12, Standard Specification for Biodiesel Fuel Blend Stock (B100) for Middle Distillate Fuels, Annual Book of ASTM Standards Vol. 05.03, 2014

[3] Knothe, Gerhard, Van Gerpen Jon Harlan, and Jurgen Krahl. The Biodiesel Handbook. Champaign, IL: AOCS, 2005. Print.

[4] "Production Statistics - Biodiesel.org." Production Statistics - Biodiesel.org. National Biodiesel Board, n.d. Web. 23 Sept. 2014. webpage: http://www.biodiesel.org/production/production-statistics

[5] Agilent application Brief:" Comprehensive GC System Based on Flow Modulation for the 7890A GC", April, 2008, 5989-8060EN

[6] O'Brien, Richard D., Walter E. Farr, and Peter J. Wan. Introduction to Fats and Oils Technology. Champaign, IL: AOCS, 2000. Print.

[7] *Ibid*., p. 27.

[8] Definition and Procedure for the Determination of the Method Detection Limit, § 40 CFR Part 136, Appendix B, Rev. 1.11. Print.

[9] McClave, James T., and Frank H. Dietrich. Statistics. San Francisco: Dellen Pub., 1991. Print.