FINAL REPORT

PROJECT TITLE:

ANALYTICAL METHODOLOGIES FOR THE DETERMINATION OF BIODIESEL ESTER PURITY - DETERMINATION OF TOTAL METHYL ESTERS

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SUBMITTED BY:

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TABLE OF CONTESTS

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I. EXECUTIVE SUMMARY

CONCLUSIONS

1) The accuracy and repeatability of commonly used gas chromatographic (GC/FID) determinations of methyl ester content of biodiesel is limited by a host of variables which need to be controlled to ensure reliable results.

Accuracy is limited by the complexity of the calibration process, the purity of substances used as standards, the changes in standards caused by handling and storage, the inability to identify and quantify all esters in the sample, the complexity of the samples themselves, for example as caused by mixtures of alkyl esters formed by compounded starting materials, and various potential interferences causing peak overlaps or misidentification.

2) Only methods capable of the highest precision and accuracy are suited for this determination.

The precision and accuracy requirements for total methyl ester analysis are high, determined by the need to distinguish 95-98% specified purity from 100% purity reliably.

3) A modified GC standard method is offered for immediate implementation as a short term stop gap. This is for determination of total methyl esters in biodiesel made from pure methanol.

A modified version of an AOCS standard method with cool on-column injection, a moderately polar megabore capillary column, autoinjection, and computer data analysis with gas chromatography/ flame ionization (GC/FID) detection is presented as the method of immediate choice to address the short term need for high precision and accurate determinations of total methyl esters by gas chromatography.

4) High performance liquid chromatography (HPLC) with mass sensitive detectors, such as evaporative light scattering (ELSD) affords a highly precise estimation of the ester content with the potential for greatly reduced calibration needs, and direct application to diesel blends and ethyl esters. However, this technology is relatively new and not yet established for biodiesel work.

5) Research is needed to improve the soundness of data from the laboratory, and reduce the effort needed to generate such data.

RECOMMENDATIONS

1. The attached procedure for determination of total methyl esters by gas chromatography (GC) should be implemented immediately. Because of idiosyncrasies associated with the GC approach this should be viewed as a short term stop gap, while research continues to find more reliable and easier to implement methods.

2. Research is needed immediately to improve the quality of total methyl ester determinations, and to find ways to reduce the effort required to obtain the desired result.

High performance liquid chromatography (HPLC) in conjunction with various mass detectors, such as the density detector, offers the potential to satisfy this need. These detectors have potential for reliable and reproducible determinations and a reduction in effort associated with calibration and interpretation, and, therefore, should be investigated thoroughly.

See also Section VII on research recommendations.

SUMMARY

The work presented here entails a review of literature concerning the analytical characterization of methyl esters of fats and oils of biological origin, followed by a laboratory study of selected methods. The purpose is to lay the foundation for a standardized procedure for routinely determining the total ester purity, and consistent with this purpose to find suitable analytical methods.

A gas chromatographic procedure is offered here for immediate implementation, which meets short term laboratory needs. This method represents a substantial improvement over earlier commonly used methods. The gas chromatographic approach is limited, however, by various idiosyncrasies, and these limitations yield a call for immediate research on promising technologies, such as HPLC with mass sensitive detectors, and ways to reduce laboratory effort to produce reliable results.

The underlying theme of the work is perhaps best summarized by the question: “What is the most economical way to obtain the accuracy and repeatability demanded by biodiesel specifications?“. The question of whether established analytical methodologies and method standards are readily adaptable to this theme is addressed here. Prospective analytical methods are first screened during the literature review using a series of preselected criteria. Among those criteria are statistical methods which help define the demands on the analytical method

for repeatability. Sound choices from the screening are further evaluated using laboratory tests to determine the repeatability (precision) and identify factors which influence accuracy.

The literature on methyl ester determinations is reviewed with a focus on chromatographic determinations of weight percent. Chromatographic methods were investigated because of limitations in the application of spectroscopic and chemical tests and the widespread *use* of chromatographic instrumentation in the biodiesel industry. Fatty acid methyl ester (FAME) analysis is the focus of much research over several decades, but the application of the results of this research to the analysis of biodiesel is relatively recent. Pioneered in the U.S., most of the specific application development of chromatographic technique to biodiesel is now best documented in the European literature. This is clearly the result of an on-going push to commercialize biodiesel there. The recent objective of establishing suitable methods for enforcing strict specifications for the weight percent total methyl ester content of biodiesel fuels differs appreciably from that of most earlier work, where the efforts are concentrated on determining the numerous specific impurities thought to pose potential or real problems.

A series of criteria are presented which were used to make preliminary evaluations of prospective methods for total methyl ester determinations. Four main criteria were used to choose methods for experimental evaluation: 1) potential for the highest attainable precision and accuracy; 2) comparable *costs* of instrumentation; 3) flexibility of instrumentation, i.e. potential application to other biodiesel applications, and other laboratory needs; 4) established technologies. Other factors, such as analysis time, operating costs, ease of method execution, calibration and standardization demands, toxicity and health hazards of handled chemicals, and the availability of instrumentation were also considered. One good possibility for HPLC detection, the density detector, was ignored because of the unavailability of instrumentation at the time of the study.

The results of the criteria evaluation indicate that gas chromatographic methods with flame ionization detection (GC/FID) offer the most immediate promise, because of the potential for high precision and accuracy, and the widespread use and popularity of GC instrumentation. HPLC with evaporative light scattering detection, a more recent technological advance, also offers the potential for highly repeatable determinations of total methyl esters, extension to biodiesel impurities, and is used as a comparison to the GC approach in the experimental evaluation. The requirements for instrumentation are presented and the costs for purchase of either HPLC or suitably equipped GC instrumentation are comparable.

Variability in the measurement process dictates a scientific understanding of the demands on analytical performance to reliably determine the purity of biodiesel, and a statistical basis is developed and presented to make appropriate method distinctions. The statistical requirements for methods capable of distinguishing between 95 or 98 and 100 % purity are presented, and these requirements dictate the need for methods with the highest precision and accuracy. Statistical measures of variability are used in the experimental evaluation of the methods.

Two GC approaches and one HPLC approach were evaluated experimentally in detail using the statistical estimates of variability (repeatability), tests of possible interferences, and the prospects for applications to blends of biodiesel with petroleum, and to ethyl esters. In each case, the methods were modified from published methods and optimized for biodiesel determinations of total methyl esters. The GC modifications of an AOCS standard focus on injection conditions optimized for biodiesel determinations, and two variations of the best possible injection conditions were evaluated.

The results show that the cool on column injection GC technique provides the best repeatability. The cool, on-column method gives a precision of plus or minus 0.34 % relative, compared to a precision of plus or minus 0.73% for the HPLC, and plus or minus 2.87% for hot on column GC. Analysis time for the HPLC approach is less than 8 minutes, whereas the total time for a GC run of the methyl esters is 40 minutes, thereby making possible more replications (which can be used to improve the statistics) for enhanced statistical reliability with the HPLC method. The HPLC method is also readily adapted to ethyl or mixed esters and esters blended with petroleum diesel. The evaluation of potential interferences such as free fatty acids, and possible application to ethyl esters or biodiesel blends shows that the HPLC appears to offer considerable advantage and flexibility.

Various factors which limit accuracy are discussed. Among those factors the availability of suitably certified reference materials, and errors in the preparation and handling of standards loom as important considerations. For the GC method, each individual methyl ester must be quantitated with a standard and this poses a serious challenge to the analyst as the complexity of the ester increases, for example, the result of mixtures of alcohols in the starting materials.

The HPLC method with mass selective detection offers potential advantages in this regard, also, with the possibility for minimal substance calibration. But the application of this technology to methyl ester purity determinations is less well established, and requires further validation and research.

Continued research is necessary to achieve reliable, cost effective approaches to the chromatographic determination of the total weight percent of the methyl ester content, and the residual glyceride impurities. Research needs (Section VII) are presented to amplify and further improve the methods and encourage the development of newer technology which promises improved method performance, reliability, and a reduction in the effort required to obtain precise and accurate results.

A draft procedure utilizing the cool on-column gas chromatography technology investigated in this study is attached.

II. INTRODUCTION

The transesterification of triglyceryl esters with simple alcohols affords an elegant pathway to materials potentially suited for diesel fuels. The starting materials for the conversion are, however, often of complex origin and lead to potential diesel products with varying extents of ester conversion, and fuel properties.

This biodiesel is comprised of numerous esters plus impurities, such as byproducts, residual reactants, free acids, and various glycerides. Such a concoction poses a challenge to analysts who need to address on one hand impurities at the 0.1% level which impact engine performance, and on the other, purity at the 95%-100% level. Both are necessary for complete fuel characterization.

The commercialization of biodiesel fuels dictates the need for enforceable specifications. The purity of the fatty acid methyl esters used in these fuels must be well defined to ensure consistent product quality and engine performance. Clearly, reliable analytical methodologies for determining biodiesel quality are needed to form a solid basis for enforcement of strict specifications of 95 % or better purity.

The following work entails a review of existing literature concerning the analytical characterization of methyl esters of oils and fats of biological origin, followed by a laboratory study of selected methods. A procedure which addresses short term needs is offered here for immediate implementation. Research needs are presented to amplify and further improve the method and encourage the development of newer technology which promises improved method performance and reliability.

III. LITERATURE REVIEW

METHYL ESTER PURITY DETERMINATIONS

The need for methyl ester purity evaluations is a two headed dragon. On one head the total methyl ester content by weight percent is needed to predict important fuel properties, and to clearly establish the identity and purity of samples which are unknown. On the other head, the level of many important impurities such as glycerol, mono and diglycerides at levels of around 0.1% (a factor of 1/1000 or less of the main ester components) or lower appears necessary for optimum engine performance (1). The two differing needs pose a serious challenge to the analyst, and while a complete scan of the impurities by one practical analytical protocol remains a lofty, yet unattained goal, separate analysis schemes for optimized precision and accuracy appear necessary.

The following summarizes the work on indirect and direct methods for determining methyl ester purity. The indirect methods are defined as those methods which attempt quantification of impurities, and the direct methods are those that determine the total methyl ester content by weight.

Chromatographic methods are the focus of much of this review, since these methods are widely used in the biodiesel industry, and gas chromatography is already specified (2,3) or proposed for ASTM.

INDIRECT METHODS FOR DETERMINING PURITY

The overall objective of indirect methods, then, is to determine all the impurities, sum them to arrive at a total impurity figure, and subtract that amount from 100 to arrive at percent purity. All of the impurities must be determined to arrive at a satisfactory determination.

Chemical, spectroscopic, and chromatographic methods are all applied to the determination of specific impurities, such as free fatty acids, soaps, water, glycerol, sterols and other unsaponifiable matter, mono and diglycerides, and residual reactants-the triglycerides and alcohol, in biodiesel. A recent monograph (4) summarizes much of the analytical methodology currently used for biodiesel in Europe, and should be consulted as a background primer on biodiesel analysis. A recent symposium addressed the need for standardized approaches to biodiesel analysis, and updated the status of several of the European Community methods (1,2). These methods or variations of them are used throughout the biodiesel industry, and chromatographic methods which allow for direct estimates of weight percent are the focus of industry.

Chemical and spectroscopic techniques are generally acceptable for biodiesel when other suitable alternatives for determining weight percent are inappropriate. These methods are based on functional group population estimates, however, and additional analytical information from complementary techniques is usually necessary to arrive at a weight percent figure. Some routine spectroscopic techniques such as mid range infrared and NMR are insufficiently sensitive or specific for determinations of many specific impurities of biodiesel at the 0.1 % range in a methyl ester matrix. Others, such as various forms of direct mass spectrometry are usually too complex and expensive for routine application in a QC environment. On the other hand, the cost of chromatographic instrumentation is well within the grasp of most QC labs and can provide direct weight percent data.

Gas Chromatography

Most of the current focus of the biodiesel community is on gas chromatographic methods, possibly due to the historical development of gas chromatographic technologies, such as the capillary column, the availability and flexibility of instrumentation, and the lure of the relatively low cost of flame ionization detectors (FID). Usually the object of gas chromatography is to separate the various molecular entities of a sample, and this is often accomplished readily with capillary GC.

Derivatization, a separate sample treatment which makes substances essentially non volatile more volatile, makes possible the determination of many of the impurities by gas chromatography. Glycerol, sterols, free fatty acids, residual alcohols, mono and diglycerides are all readily derivatized with the silylating agent BSTFA (5), and this forms the basis of current GC methods for impurity determinations.

Freedman et al.(6) published the classical basis for determining BSTFA derivatized mono and diglycerides and residual triglycerides as a means for rapid monitoring of biodiesel transesterification process mixtures by GC. Several modifications of this method for biodiesel analysis have appeared, differing in column stationary phase, length and diameter, injection parameters, internal and external standardization procedures (7-11).

An AOCS standard,Cd 11b-91 (II), describes the determination of mono and diglycerides, based on BSTFA derivatization. Bandioli et al. (8) investigated the use of cool on column injection techniques, and this is incorporated into the method presented by Plank (10) for biodiesel. Plank makes a critical assessment of methods used for determining the acylglycerol (mono, di, and triglycerides) content of biodiesel fuels at the 0.1% level by gas chromatography. BSTFA derivatization methods are also reported for determinations of glycerol (12) and residual methanol (13) in biodiesel FAME.

High Performance Liquid Chromatography (HPLC)

The HPLC approach to analysis of lipids has blossomed in the last ten years. Advances in HPLC detection have resulted in renewed interest in this technique for determination of lipid impurities such as the mono, di and triglycerides (14). Specific applications to biodiesel, however, are few.

Rather than separate the lipid impurities into individual compounds and determine each entity, the emphasis of much work is directed at separation of lipids into classes of compounds, such as proposed by Christie (15) using evaporative light scattering detection. This HPLC approach differs from that of GC where each compound is separated and determined individually. The esters as a group of substances are separated from the various glyceride compounds. The lure is the potential simplification of the analytical calibration process to but a few compounds, with the possibility of universal calibration.

Examples of applications of HPLC to biodiesel are published. Bruns (16) studied the application of early evaporative light scatter detection (ELSD) technology to the determination of mono, di and triglycerides in methyl esters. The ELSD offers high sensitivity for low vapor pressure substances, such as those found in biodiesel. Trathnigg and Mittlebach used density detection (17) to monitor transesterification reactions for biodiesel production.

Thin Layer Chromatography (TLC/FID)

Thin layer chromatography (TLC) with flame ionization detection is used for classical class type separations in lipid analysis (18), and can form the basis of quantitative estimates. Manual sample application to the sample plates is exacting for the best quantitative work, and a robot may be needed to do this on a routine basis. Limits of detectability are reported in the range of tenths of a percent, which is considerably higher than that required for routine monitoring of glyceride impurities at the 0.1% level in the European Community nations.

TOTAL METHYL ESTER DETERMINATIONS

Introduction

The analysis of methyl esters of fatty acids is the subject of extensive research over several decades. The focus of that research is on detailed investigations of the composition of triglycerides, and other lipid (fat) components in living systems, food and industrial oils. The objective of the analytical research is mainly to define the type of fatty acid groups attached to the glycerol backbone of fats and biogenically derived oils.

Availability of Standard Procedures

Many standard analytical procedures are published for the characterization of fats and oils by the AOCS (American Oil Chemists Society) AOAC (Association of Official Analytical Chemists) and ASTM, and these are applied to numerous different analytical challenges with varying degrees of success. Gas chromatography forms the basis of much of the current analytical methodology for biodiesel, and methods used for determining the total methyl ester content are based on these standards, particularly those published by the AOCS.

Weight Percent and Functional Group Methodologies

A whole spectrum of organic analytical techniques is applied to fatty acid methyl ester characterization. While many different methodologies are available, these can be roughly categorized into two groups. In the first, the total methyl esters are determined, and weight percent is derived directly from weight standards, as is done in various chromatographic techniques. In the second, the weight percent is indirectly derived from an estimation of the population of functional groups (functional groups are the specific chemical building blocks of a molecule which determine molecular properties) on a substance's molecular framework.

The application of functional group methodologies is complicated by the fact that biodiesel represents numerous substances somewhat similar rather than a distinct single substance. Without additional detailed analytical information about impurities and chain length distribution, the accuracy of the weight percent estimates in the functional group tests is generally limited. While acceptable in many other instances the high degree of precision and accuracy required for biodiesel determinations limits the utility of functional group methods. Chromatographic separation which defines the specific chemical composition can greatly enhance the value of spectroscopic techniques.

Mid range infrared (IR) and nuclear magnetic resonance (NMR)are examples of widely used spectrometric techniques which focus in on the functional groups of a substance. Various chemical tests such as saponification number or acid number are aimed at functional group populations, also.

Chromatography

Chromatographic methods, such as gas chromatography (GC), high performance liquid chromatography (HPLC) and thin layer chromatography (TLC) separate the many individual compounds of families of substances such as those found in biodiesel. These separation tools are applied widely to the characterization of fatty acid methyl esters, oils and fats, and are the most useful in obtaining quantitative determinations of weight percent directly.

Only relatively recently is there a concerted effort to refine analytical methods for the purpose of defining the purity of the methyl esters of fatty acids used in diesel fuel substitutes (biodiesel). The starting point of many of these methods is the gas chromatographic separation work used to define the fatty acids attached to triglycerides, or of free fatty acids, or the makeup of various mono and diglyceride substances, by conversion of the fatty acid residues to fatty acid methyl esters.

Methods which specifically address the analytical rigors required to meet the tight proposed specifications for the total purity of biodiesel fuels by gas chromatography, the most widely used technology to date, are unavailable. Newer technologies offer the possibility for reduced tedium to achieve appropriate standardization and calibration. Given the complexity of these analyses, that possibility, if realized satisfactorily, would offer a considerable advantage.

Gas Chromatographic (GC) Methods

The application of gas chromatography to the analysis of methyl esters is extensive. Already mentioned is the early application of this technique to the characterization of oils and fats.

Samples of oil or fat are typically completely saponified (converted to a fatty acid soap) and then converted to the methyl ester by a derivatization reaction with a substance such as boron trifluoride in methanol. The methyl esters are then analyzed using a capillary column and flame ionization detection. This is a standard approach to deriving the fatty acid distribution and is the basis for methods published by ASTM, AOAC and AOCS. Typically, this approach is used to amplify the meaning of simple tests which determine the total fat content by extractions. These GC methods form the basis of current efforts to determine the total methyl ester content of biodiesel (19-22).

Several approaches to the gas chromatographic determination of the total methyl esters are reported. These differ primarily on the basis of resolution, accuracy and precision, the types of columns and detectors available.

COLUMNS AND INJECTOR TYPES

Both non polar and polar columns are used depending on the particular advantages required for a specific determination. A challenge with non polar types such as the 5% phenyl/dimethyl silicone phases is the separation of the main types of Cl8 fatty acid methyl esters in many vegetable oils, i.e. the stearate, oleate, linoleate, and linolenate. Such non-polar columns, which are widely used for determinations of ester impurities by BSTFA derivatization, make quantification difficult, and are inappropriate where the highest precision and accuracy are required.

Short columns for low resolution requirements are evaluated. A low resolution GC/FID method was developed by Freedman et al. (6) for the purpose of monitoring transesterifications of soybean oils for the conversion to biodiesel. The column has a non polar stationary phase with split injection.

The AOCS standard methods Ce 1-62 (20), and ASTM method D 1983-90 (19) are methods for determining fatty acid composition by GLC of methyl esters with packed column technology. Ackman (23) has commented on the advantages of capillary columns compared to packed columns. Ce le-91 (22) and Ce lc-89 (21) determine the fatty acid composition by capillary GC techniques. Both capillary methods employ split injection. Method Ce le-91 utilizes a polar cross bonded polyethylene glycol stationary phase with split or heated on-column injection. The Ce lc-89 standard method for determining the cis/trans unsaturation ratios and unsaturation positional isomers of fatty acids (as their methyl esters) utilizes a highly polar cyanopropyl column, with a split injection technique.

Non polar, so-called "boiling point" columns are also used by some investigators for methyl ester separations, a possible advantage when chain branching of the hydrocarbon backbone requires investigation. These columns have difficulty in separating the important unsaturated methyl esters such as oleic, linoleic. and linolenic.

QUANTITATION

Slover and Lanza (24) have published a detailed study on factors which affect the accuracy and precision associated with the quantitative analysis of food fatty acids by capillary gas chromatography of methyl esters using the split injection technique. Their study includes the results of calibration work with correction factors. The data indicate that the correction factors vary in a complex way on concentration of analyte and the chemical structure of the analyte. The correction factors must be calculated for each analysis system, as a significant portion of the correction is the result of analyte decomposition and irreversible adsorption to the active sites in the sample flow path.

HPLC

Several methods are reported for determining the total methyl ester content of biodiesel. A precision and accuracy of plus or minus 1% are reported (17,25). Trathnigg and Mittlebach (17) investigated separating the biodiesel into its esters and impurities using a combination of a cyanopropyl bonded phase column and a styrene divinyibenzene column with a density detector and chloroform mobile phase.

ANALYTICAL PRACTICES IN THE US

In a study hampered by confidentiality concerns, the current practices of industrial firms manufacturing biodiesel in the U.S. were surveyed. The results of this study indicate that the methods currently in use for impurities are based on BSTFA derivatization, and AOCS standards for iodine value, acid number, and glycerol, and AOCS standards Ce le-91 for methyl ester distribution, and total methyl ester determinations.

IV. STATISTICAL REQUIREMENTS

ACCURACY AND PRECISION

Summary

The accuracy and precision requirements for the various methods are examined here. This is not a comprehensive review, but rather an attempt to determine approximate boundary parameters, so that methods are more easily evaluated. Because chromatographic methods are the subject of the evaluations in the coming sections they are the focus of this discussion.

While the accuracy of a determination is related to various sample related considerations and is difficult to predict, a useful question to pose for the total methyl ester analysis is "what is the maximum allowable precision(standard deviation) which makes possible a viable distinction between 100% pure total methyl ester and the minimum allowable purity (e.g. a proposed specification of 98% purity)?". This information allows for an informed decision of the minimum requirements for precision and accuracy for the methodology, and also the sample analysis replication requirements.

The estimated standard deviation associated with a particular methodology is a statistical statement of the performance of a method and its precision. Significance testing utilizing t statistics and confidence limits was used here to estimate the maximum standard deviations allowed to distinguish the difference between 100% purity and other levels of purity at different probabilities (9/10, 95/100, and 99/100) and replications. The ability to distinguish between pure 100% methyl ester and two different levels of purity, 98 and 95%, was calculated.

Method Precision Requirements

Tables 1 and 2 list the estimated maximum allowable method errors in terms of the standard deviation. The number of determinations for each of both pure, 100 % standard, and sample required to achieve a particular standard deviation is n, while the p is a probabalistic confidence level. If the actual standard deviation for a single determination is greater than or equal to that appearing in the table then the difference between the true means of sample and standard might be zero at a particular confidence interval.

TABLE 1

MAXIMUM ALLOWABLE STANDARD DEVIATION FOR DISTINGUISHING DIFFERENCES BETWEEN 98% AND 100% PURITY

NUMBER OF REPLICATES EACH FOR SAMPLE AND STANDARD

N=	2	3	4	
CONFIDENCE LEVEL PROBABILITY P	STANDARD DEVIATION PLUS OR MINUS PERCENT			
9/10 (90%)	0.68	1.15	1.46	1.70
19/20 (95%)	0.46	0.88	1.16	1.37
99/100 (99%)	0.20	0.53	0.76	0.94

TABLE 2

MAXIMUM ALLOWABLE STANDARD DEVIATION FOR DISTINGUISHING DIFFERENCES BETWEEN 95% AND 100% PURITY

NUMBER OF REPLICATES EACH FOR SAMPLE AND STANDARD

N=	2	3	4	5
CONFIDENCE LEVEL PROBABILITY P	MAXIMUM ALLOWABLE STANDARD DEVIATION PLUS OR MINUS PERCENT			
9/10 (90%)	1.71	2.87	3.64	4.25
19/20 (95%)	1.16	2.21	2.84	3.43
99/100 (99%)	0.50	1.33	1.91	2.36

A quick review of the tables reveals that the requirements for distinguishing between 95 and 100 percent are considerably less demanding than those for distinguishing between 98 and 100%. For example, a precision of less than plus or minus 1.16% is needed for a 19/20 confidence (95%) level to distinguish between 95 and 100% by analysis of duplicated samples and standards. In contrast, a precision of less than plus or minus 0.46% is necessary to distinguish 98 and 100 percent with duplicate analyses of samples and standards.

Stated in another way a method with a precision of plus or minus 1.15 % would require quadruplicate determinations of both standards and samples to achieve 95% confidence of a difference between 98 and 100%, and just duplicate samples for the 95% confidence of a true difference between 95% and 100%. This is hypothetical, in that it assumes that in the analysis of actual samples there are no biases (these are some), and that the precision of the determination of the samples and standards is identical (which should be true,but there are no guarantees). That is, the real situation is worse taking into account biases, and sample related measurement variability.

ACCURACY

Factors affecting the accuracy of methods for the determination of total methyl esters include the following:

1) The precision of the method.

2) The ability to quantitate overlapping peaks.

3) Interferences from unknown substances

4) Accurate assignment of peaks including minor peaks, and peaks less than 1%.

5) The inertness of the sample flowpath during chromatographic separation.

6) The accuracy of certifications of standards.

Because of the small differences between 100% and 95% pure substances the need to control these factors is acute.

1) Precision- in absence of analytical biases which alter the analytical result, precision determines accuracy. High precision, as discussed above, is key for determining the success of a method, and becomes critical on a practical level, when the replication needed to have a certain degree of confidence is considered. Put in another way, the rate of sample throughput, eventually limits the degree of replication possible for a given method.

2) The ability to quantitate overlapping peaks is especially important if the response factors of the two overlapping ester peaks differ. The variation of response factors as a complicated function of chemical structure and concentration is discussed in a previous section. To distinguish between 98% pure and 100% pure samples dictates special care for the main and minor components. Resolution, or the ability to separate the main components chromatographically is critical.

Eliminated from further consideration here are methods which fail to resolve the main Cl8 fatty acid methyl esters, such as gas chromatographic methods based on polydimethylsilicone stationary phases used in BSTFA derivatization methods.

Accurate quantification of overlapping peaks is becoming more of a concern to fatty methyl ester analysts and to achieve the best possible quantification of often complex chromatograms computers with data processing techniques are required. Possibly, more advanced techniques of detection, such as GC/MS are necessary to analyze biodiesel made from mixtures of alcohols.

3) Interferences defined here are substances which are not fatty acid esters. Peak overlap with methyl esters is a potential concern, as is spurious high or low level peaks, which in the latter case might lead to several peaks at levels of less than 1% -easy to ignore, or to mistakenly consider as methyl esters.

In GC, any peak which is unidentified is a potential interference because the whole chromatogram is scanned. Some possible interferences include fatty acids and some hydrocarbons.

4) The identity of peaks is normally accomplished through a comparison of retention times of the components of standards to those of the unknown sample. Unidentified esters go uncounted, and the presence of minor amounts of alcohols in addition to methyl could cause low estimates of ester content.

As an example. two or three small unidentified peaks just less than 1% could determine whether a fuel passes or fails specifications for 98% purity. Peaks down to 0.1% need to be identified and quantified to meet such a specification.

5) The inertness of the sample flowpath is particularly important in chromatography because microscopic amounts of

substances are introduced to the analysis system, and to ensure that every component passes through to the detector is extremely important.

Problems associated with adsorption, destruction of substances are particularly acute with gas chromatography. The inlet of the gas chromatograph is susceptible to such problems because of the high temperatures of injection, and residues left behind from previous samples. Unsaturated methyl esters are particularly susceptible because they are readily oxidized at higher temperatures. The analysis system must be inert.

6) The availability of certified reference standards of sufficient quality is a key requirement for accurate results. Vendors often provide certificates of analysis. Once such standards are in hand adequate procedures to maintain the purity of the standards is necessary.

Many warnings are issued regarding the susceptibility of the methyl esters to light, heat and oxygen. The degree to which this impacts ester analysis is unclear. Some compounds, such as the highly unsaturated compounds, are relatively unstable and reactive towards oxygen. However, relating this information to routine practice in the laboratory to avoid losses of even 1 or 2%, which is likely to be acceptable in many other applications, is difficult.

As yet, NIST has no certified reference materials for methyl ester determinations. Mixtures of esters are available from NIH and AOCS through private standards companies. Private companies are the biggest source of standard materials. The companies sometimes provide a certificate of analysis to accompany the sold material. There are, however, no independent checks on the quality of these materials, and provisions are lacking in standard methods to check the purity in the laboratory, where changes in the purity by esposure to air, heat or light are likely.

One such company was contacted to determine the level of variation in purity of pure substances and mixtures used for standards. The pure materials are sold as >99 % purity, and are checked by gas chromatography and thin layer chromatography prior to customer delivery. These tests may be insufficient to define purity adequately.

METHODS EVALUATIONS

INTRODUCTION

To routinely distinguish with confidence pure 100% methyl esters from 95-98% purity requires the highest attainable accuracy and precision. Based on this need, two modified methodologies capable of the highest precision and accuracy were chosen to determine which is best suited for routine determinations of biodiesel purity. These methodologies were based on gas chromatography (GC) and high performance liquid chromatography (HPLC), and were optimized for biodiesel needs. Two variations of injection conditions of the GC approach were tested. The results show that GC with direct, cool, on column injection provides the best precision.

CRITERIA FOR METHOD SELECTION

The following criteria were considered in selection of methods:

*Potential for the highest attainable precision and accuracy
*Technology status-state of technology development
*Analysis time/sample throughput
*Flexibility of required instrumentation/potential adaptability
    to other biodiesel analyses
*Operating costs
    Maintenance of equipment
    Chemicals/gases
    Degree of training
    Man time
"Method performance-precision/accuracy
*Capital costs of instrumentation
*Instrumentation availability
*Toxicity and health hazards of handled chemicals
"-'Ease of method execution/degree of tedium
*Calibration and standardization demands

RATIONALE

Four main criteria were used to choose methods for experimental evaluation:

1) Potential for the highest attainable precision and accuracy as described in the statistical requirements previously discussed.

2) The comparable costs for a new GC/FID and isocratic HPLC/ELSD are similar and within the range of 35-$40,000 sourced from high profile vendors. These instruments are equipped in a way which makes them suitable for the high precision work required here. These costs only reflect those associated with the instruments, and do not reflect any facilities modifications necessary to accommodate the instruments. More sophisticated and expensive detection instruments such as FTIR or MS which presumably offer more specificity were not considered because of substantial increases in costs and manpower skills.

3) The flexibility of instrumentation, i.e. potential applications to other biodiesel analyses, and other laboratory needs.

4) Established methodologies- gas chromatography/flame ionization (GC/FID) is already used widely for biodiesel analysis, is already specified as the technique of choice for impurity analysis, and is the heart of proposed ASTM specifications for biodiesel analysis.

The potential for the highest attainable precision and accuracy and the widespread use and popularity of GC/FID instrumentation in biodiesel analytical laboratories are the most heavily weighted criteria above. The GC/FID approach is, therefore, the focus of this evaluation. HPLC with recent ELSD detection technology offers the potential for highly precise determinations of methyl esters, extension to biodiesel impurities, and is used as a comparison to the GC approach.

GC METHODS

The highest precision and accuracy attainable by GC is afforded by direct on-column injection techniques (27). In particular, cool on-column technologies avoid common GC pitfalls associated with hot injection, such as needle discrimination, degradation of easily oxidized or thermally degraded components, and sample component discrimination, all of which impact accuracy and precision negatively. The sample is placed directly into the column, rather than onto insert liners with inertness which is questionable. Questions regarding the accuracy of splitting, a common GC injection technique for methyl esters, are avoided with the on-column approach. The potential for sample discrimination effects is greatly reduced by the on-column approach, and that gives this technique the best chance for high laboratory repeatability and interlaboratory reproducibility.

Cool, direct on-column and heated-on column injection techniques were evaluated by repetitive analyses of a methyl ester mixture similar to that derived from soy transesterification to determine precision. Although cool, on-column injection is intuitively preferable, some advantage may be realized for certain older instruments with packed column inlets which are readily converted to direct, heated, on-column injection. Because the simplest, least possible interactive sample flow path in the injection zone was sought, split injection was not included in the evaluation. Modifications of AOCS method Ce-le-91, a capillary GC method for determining the fatty acids in edible oils and fats (by analysis of their methyl esters), were used for the evaluation. since no suitable alternative procedure could be found in the public domain for determining methyl esters which would meet the precision and accuracy requirements of biodiesel analysis.

The modifications of this method are as follows:

1) The injector is direct on-column (this is an option of AOCS method Ce-le-91, but only the split injection method is provided therein).

2) The column is a 30 M 0.53mm megabore with 1.0 urn film (we are sacrificing some resolution to : a) gain capacity for higher working concentrations, which allows for better quantification of minor and trace peaks, and higher peak areas for major peaks); b) to simplify the sample flow path, and allow for direct needle insertion into the column. This avoids usage of retention gaps which can complicate the injection process.

3) Helium is the carrier gas (caution: Helium is an asphyxiant). Helium is an option of Ce-le-91.

4) The column temperature program is modified to accommodate 2) and 3) above.

HPLC METHOD

A precision of plus or minus 1% is reported for analyses of biodiesel by HPLC (17,25), and, therefore, HPLC is potentially suited for routine biodiesel analyses of total methyl esters. The HPLC approach offers better control of injection conditions, where many of the problems of GC are encountered. Loop injectors, for example, allow for manual, highly controlled, and repeatable injection volumes, and thermal or oxidative degradation of the sample during injection is far less of a concern than in GC.

Among methods considered were those employing refractive index, density, flame ionization and evaporative light scatter detection. Refractive index detection of the separated components is highly dependent on the response factors of the individual methyl esters (17), and FID detection, at best, offers limitations in response factors, as in GC/FID.

Density detectors used in a previous study (17) were not marketed commercially for HPLC in the U.S. during the time of this study, and therefore, were removed from consideration. The toxicity of mobile phase solvent components was also considered and halogenated solvents, such as chloroform, were deemed less preferable than hydrocarbon solvents in this regard, and methods using such solvents were given low priority.

A modification of the methods by W. W. Christie (15) and Bruns(16) were used to evaluate the use of evaporative light scattering detection (ELSD). In these methods, classes of compounds, such as mono, di and triglycerides, similar to those found as impurities in biodiesel, are separated by normal phase chromatography with hydrocarbon solvents. The families of compound classes emerge as single or but a few peaks when compared to the many peaks which emerge from a high resolution GC column.

This approach offers promise in extensions to biodiesel applications, and the potential advantage of reduced calibration, since the highly sensitive detector responds directly to changes in mass, a universal indicator. Initial studies of biodiesel showed that the methyl esters emerge from the column as one peak, rather than five or more, which is clearly a positive sign for a significant advantage over high resolution, and individual peak identification and quantitation.

The modifications are as follows:

1) The method is applied to methyl esters of fatty acids, as first reported by Bruns (16).

2) The solvent mobile phase is isocratic, i.e. is constant, rather than a more complicated gradient form. Conditions for the separation of hydrocarbons, methyl esters and triglycerides were developed using hydrocarbon solvents such as hexane or pentane.

3) The detector is recent technology.

COMPARISON OF INSTRUMENTATION REQUIREMENTS FOR GC AND HPLC

The cost of instrumentation requirements for an isocratic HPLC with ELSD to do these methods are comparable to those for the GC/FID with accessories, and in the range of $ 35-40,000. Table 3 lists various requirements for each instrument.

A difference in requirements between the instruments is in the sophistication of the injection equipment and the computerized data analysis sophistication. To achieve adequate precision, a manual injection loop is possible for HPLC, and the autoinjector is optional (though desirable). For GC, however, the autoinjector is necessary for optimum precision. Because of the simplified chromatogram of the HPLC compared to the high resolution GC, only a good recording integrator is absolutely necessary. In GC, the complicated tracings of the chromatogram dictate the highest flexibility for data analysis.

EXPERIMENTAL

Standard Solutions

Pure standards, standard mixes and samples of the methyl esters were prepared for analysis by weighing approximately 100.0 mg of the pure standards in a 10 ml volumetric flask, and dilution with hexane. Approximately 1 mg/ml dilutions were prepared for routine injections by 1:10 dilution.

Evaluation of methyl undecanoate internal standards was performed on stock solutions prepared as above with an additional 40.0 mg of internal standard material.

TABLE 3
INSTRUMENTATION REQUIREMENT DESCRIPTION

	GC	HPLC
INJECTION	Autoinjector High Precision Syringe Cool, direct on-column	Manual valve Loop
DETECTION	Flame ionization	Evaporative light scatter
DATA HANDLING	Computer w/chromatography software/ data handling	Recording integrator
OVEN	Standard, high precision oven, T maximum at least 280 degrees C	None
PUMP	None	High Precision, pulseless HPLC capable of 3.0 ml/Min.
MISCELLANEOUS	carrier gas purifiers to less than 2 ppm oxygen and moisture.	detector gas filters

Instrumentation and Operating Conditions

Gas Chromatograph

Analyses were performed with a Varian 3400 equipped with flame ionization, a packed injector modified for direct capillary on-column injection, and a computer data station. The column was a moderately polar, 0.53mm megabore column with 1.0 um film, as described in the attached draft procedure. The injector temperature was either 250 degrees C for the experiments with heated, on-columm, or 50 degrees for experiments with cool, on-column injection. The design of the cool on column variation described here is inadequate in a high throughput lab, because of the excessive time required to achieve the set injector temperatures between runs. A more conventional cool, on-column design is necessary for routine work. Detector temperature was 250 degrees C. Helium was used as carrier gas at a flow rate of 5.0 ml per minute. Detector gases were set according to manufacturer recommendations.

Samples (2 ul) were injected manually into the column using a syringe with a 4 inch needle to place the sample at an adequate distance away from the cool inlet to prevent recondensation of sample in the inlet. Heated injector samples were introduced with a collar fitted with the 4 inch syringe to allow for injection into the heated area of the injection port with the same syringe. A smooth, rapid depression of the plunger was used for all injections. The column was started at 40 degrees and then heated at 15 degrees/minute to 120 degrees, 4 degrees per minute to 250 degrees, then held for 2 minutes. Total run time was about 40 minutes.

Liquid Chromatograph

Analysis was performed using a Waters 510 pump with a Rheodyne 7125 loop injector and a 5 ul injection loop. The detector was a Sedex 55 evaporative light scattering unit, and the output was sent to a computer data station. The detector was modified to allow for room temperature cooling and routine operating temperatures just above 22 degrees.. The nitrogen flow rate was adjusted with a pneumatic pressure gauge set at 2.1. The column was a 5um cyanopropyl bonded phase 25 cm x 4 mm ID. Flow rate of hexane or pentane was 0.8 ml./minute. Total run time was 6-8 minutes.

GC RESULTS

Example GC Chromatograms

Figure 1 shows the separation of methyl esters typical of those found in a transesterified soy sample (Mixture G). The main peaks are those of the three main C18 methyl esters of stearic. oleic, and linoleic acids. Also present are the C16 methyl esters of palmitic and palmitoleic acids and a small amount of C14 methyl ester of myristic acid. Figure 2 shows the separation of a standard material containing higher C22 and C24 methyl esters. as well.

INJECTION TECHNIQUE

Comuarison of Hot and Cold On-Column GC Injection Techniques

The precision of determining the total methyl ester content of the mixture depicted in Figure 1 was calculated from the standard deviations associated with measurements of individual esters. This figure of merit is the square root of the sum of the variances for each ester. Table 4 lists the different compounds in the samples and their respective standard deviation for both hot and cold on-column injection. Ester 16-O was used as an internal standard to correct for variations in injection volume, and consequently was not listed.

The results show that the precision of the cool injection is more favorable to repeatable determinations than the hot variation. This result was not a surprise because of the many known vagaries associated with hot injection, as already discussed. These results clearly suggest that cool on column techniques afford an advantage over hot injection.

Hot injection is, consequently. not recommended for determinations of total methyl esters in biodiesel.

FIGURE 1
GC CHROMATOGRAM TRACE OF MIXTURE G METHYL ESTERS

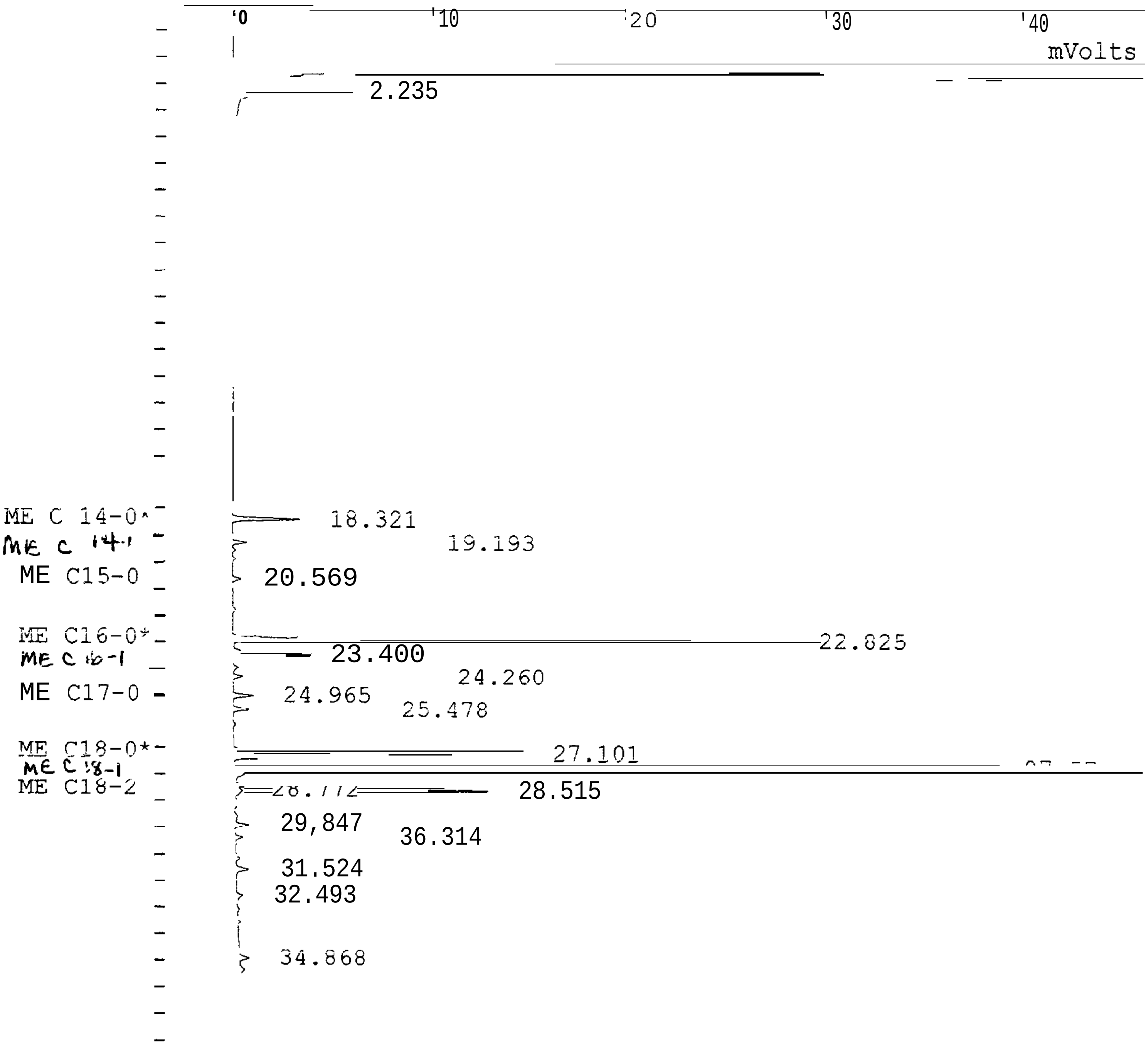
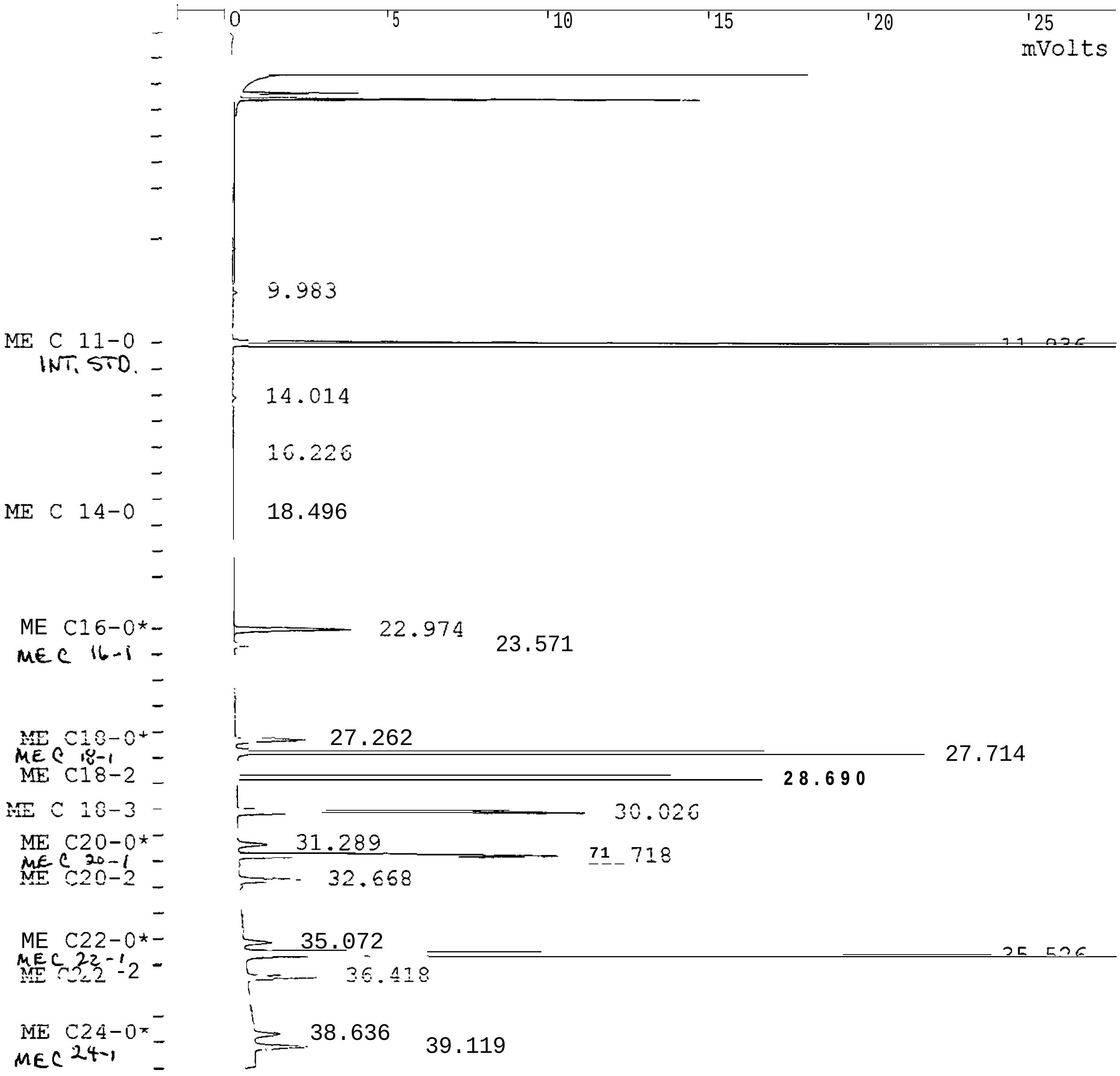


FIGURE 2
GC CHROMATOGRAM TRACE OF STANDARD MIXTURE



Internal Standards

A review of possible internal standards was undertaken. The most widely used internal standards for methyl ester analysis are odd carbon methyl esters. Apparently, such esters are sometimes present at measurable levels in manufactured methyl esters for biodiesel, and, therefore, only one ester, methyl undecanoate, is potentially a suitable internal standard (26). The precision of the cold on column technique was compared using the C11 methyl ester and a Cl6 ester inherent to the sample. The results of that test indicate that the precision of the method is adversely affected 'by the Cl1 ester, and, therefore, the use of an internal standard is, at this time, not recommended.

HPLC RESULTS

Example of HPLC Chromatograms

The HPLC/ELSD trace of a mixture of methyl esters is shown in Figure 3. A key feature of these chromatograms is the single peak containing the fatty acid methyl esters. This peak is clearly resolved from any hydrocarbon or glyceride contaminants in the sample. Hydrocarbons intentionally added in the form of mineral oil emerge from the column well ahead of the ester peak, and the neutral glyceride series begins to emerge well after the methyl ester peak. Continuous runs of 7 to 8 minutes were adequate to fuily quantitate the methyl ester peak.

Evaluation of Precision

The precision of the method was estimated by manual replicate injections. The sample injected was the same as that used for evaluation of GC precision consisting of methyl esters similar to those of a preparation from soy oil. The estimated relative standard deviation with seven replicates (N=7) is plus or minus 0.73%. This shows that the method is capable of good precision, and with an analysis time of just 7 minutes, capable of the rapid replication required for improving precision of the mean.

GC INTERFERENCES

Possible interferences in the determination of the total methyl ester content were evaluated. Among those potential interferences are free fatty acids, commonly found as impurities,

FIGURE 3
HPLC/ELSD CHROMATOGRAM OF METHYL ESTER MIXTURE G

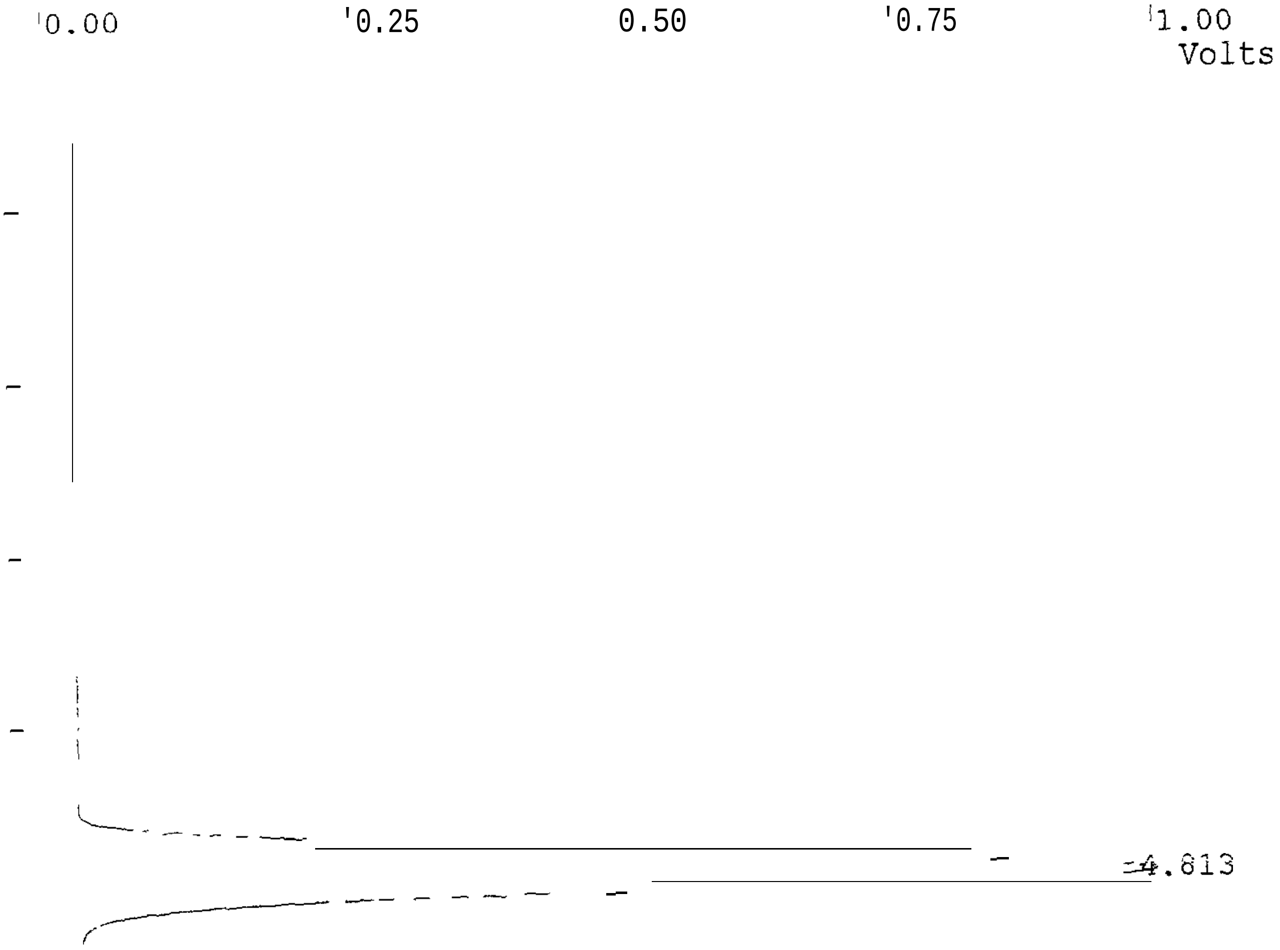
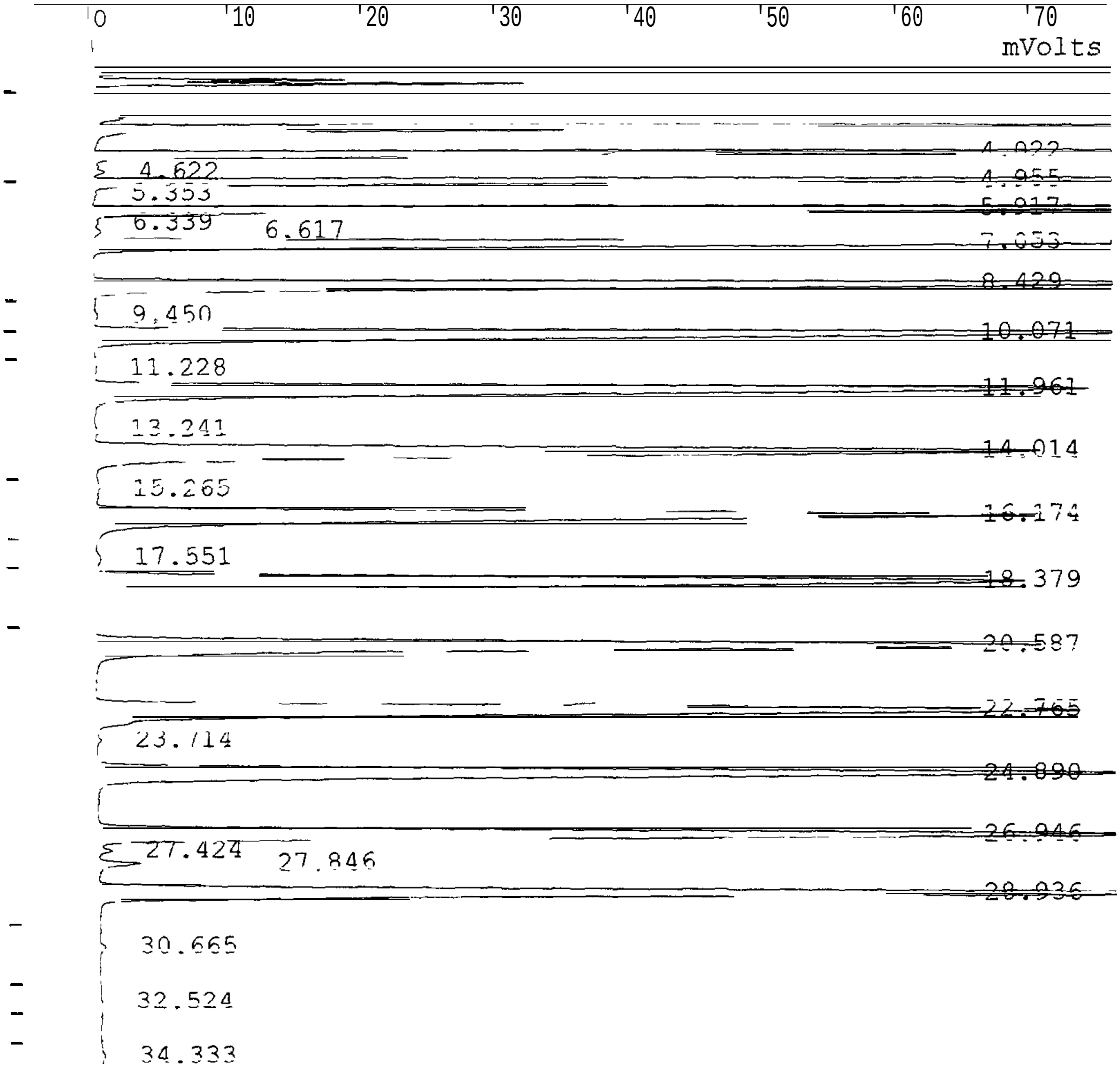


FIGURE 4
GC CHROMATOGRAM OF HYDROCARBONS SIMULATING DIESEL



and hydrocarbons, such as those associated with diesel fuel. This evaluation is not all inclusive, and any substance which elutes during the emergence of the fatty acid methyl esters is a potential confounding factor, if unidentified, or unresolved from the methyl ester peaks.

Figure 4 shows a GC chromatogram trace of a mixture of C10-C25 hydrocarbons similar to those found in diesel fuel. A regular pattern of peaks appears over a considerable range of the methyl ester series. These peaks are broader than those of the esters, and occur near or directly on top of several ester peaks. The breadth of compounds found in diesel is actually much larger than those represented in the chromatogram, and clusters of peaks so close in characteristics are usually found in diesel. Clearly, diesel or similar hydrocarbon contaminants would pose a quantification interference.

Free fatty acids are frequent impurities in biodiesel, and can be chromatographed. Figure 5 shows a GC chromatogram trace of the three free fatty acids, myristic, stearic and oleic. Free fatty acids are much less volatile than the corresponding methyl esters, but nonetheless can emerge in a region of methyl esters. The oleic and stearic acids emerge from the column after the C24 methyl esters, but the myristic (C14) acid emerges in a region near to the C20-2 ester. By mathematical inference (which is legitimate because of the predictability of fatty acid elution) the Cl6 acid cluster emerges near the C22-2 methyl ester, and the Cl2 acids, near the C18-2 ester. The acid peaks have much less than perfect shapes with considerable tailing on the downside, and, therefore, can pose a mathematical challenge to automatically correct peak overlaps accurately.

POTENTIAL APPLICATION TO DIESEL FUEL BLENDS

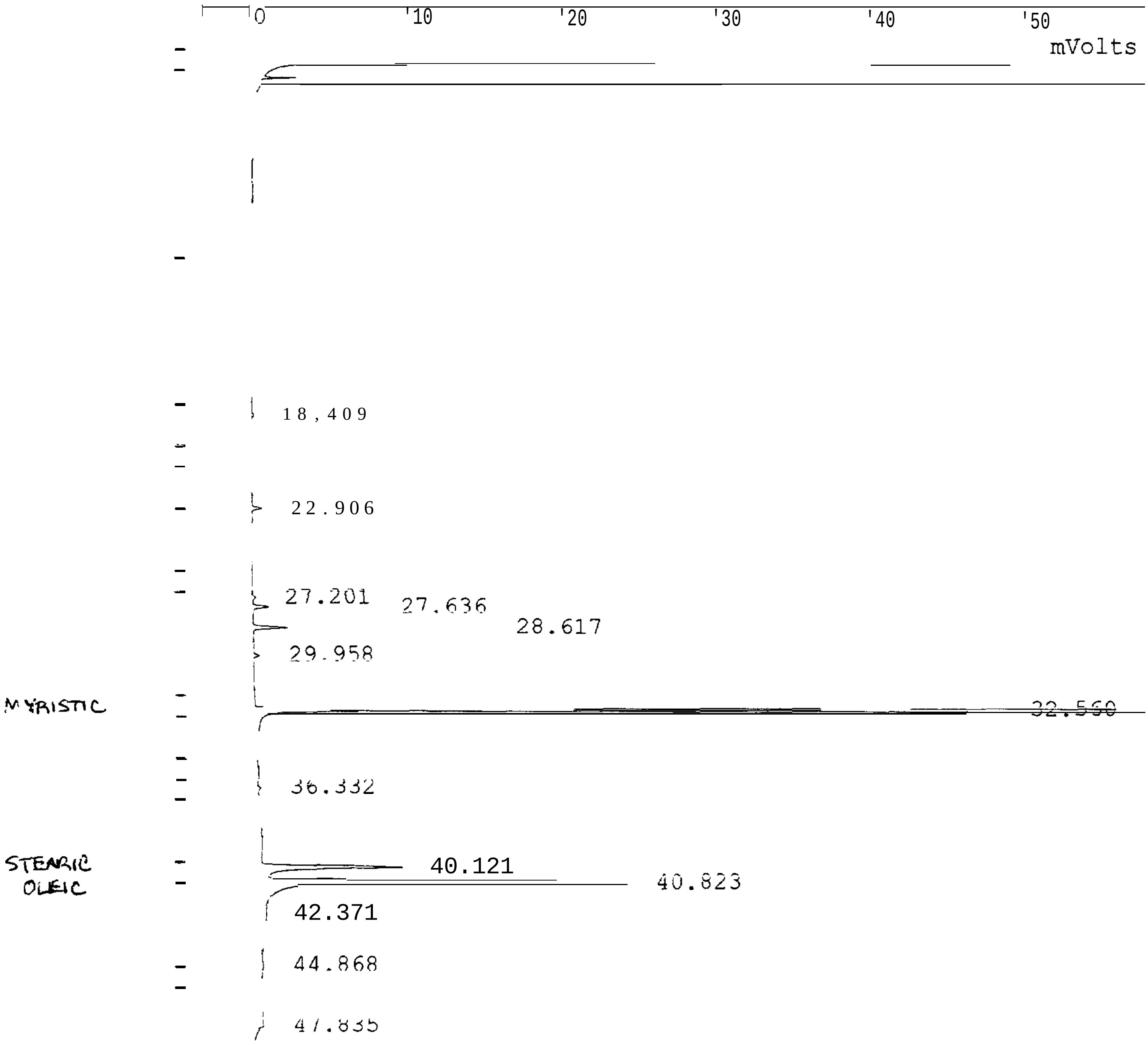
GC/FID

The potential application of the GC method to diesel fuel blends is complicated by the complexity of hydrocarbons found in diesel, many of which could elute in the range of methyl esters as is shown in Figure 4. Biodiesel blends of 20-30 % would probably reauire reliable sample pretreatment measures to separate the esters from the hydrocarbon components.

FIGURE 5

GC CHROMATOGRAM OF FREE FATTY ACIDS

MYRISTIC, STEARIC, OLEIC



HPLC/ELSD

The methyl esters are well separated from hydrocarbons which are likely in diesel fuels. As an example, a trace of mineral oil added to methyl ester mixture G emerges almost a minute before the methyl ester peak.

POTENTIAL APPLICATION TO ETHYL ESTERS

GC/FID

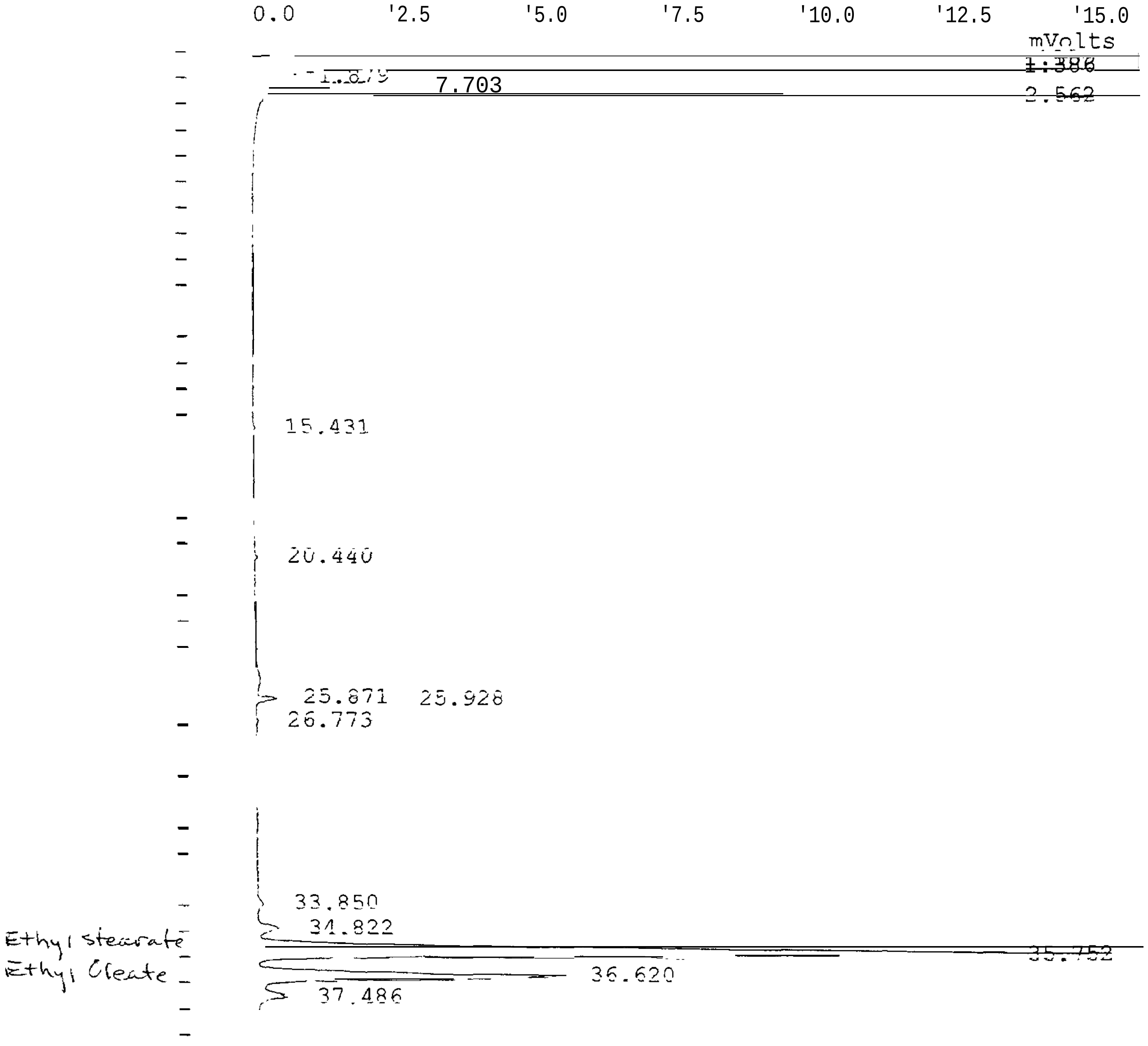
When ethanol is the starting material (or one of the starting materials) ethyl esters are formed with properties which differ substantially from those of the methyl esters. To determine the degree of success the method proposed here applies to ethyl esters the retention characteristics of two ethyl esters, the stearate and oleate were analyzed and compared to the results of methyl esters. The results show that the method would require modification to address ethyl esters.

Figure 6 shows a GC chromatogram of the ethyl stearate and oleate. The peaks at retention times, 35.75 and 36.62, respectively show that these esters are clearly separated from one another. These times are very close to those of the C22-1 and C22-2 methyl esters. Also, compared to the retention times of the methyl esters (27.3 and 27.7 minutes) these are considerably longer, and, in fact, close to the end of the standard run. In a normal run of 40 minutes the likelihood of loss of C22 and higher ethyl ester data is high. Clearly the GC method will require modification to accommodate the ethyl esters.

HPLC/ELSD

The analysis of ethyl stearate/oleate mixtures leads to a single peak in the area of the methyl ester mixture peaks, and possibly a slight shift to lower retention times.

FIGURE 6
GC CHROMATOGRAM OF ETHYL STEARATE AND ETHYL OLEATE



VI. RESEARCH NEEDS

1) PROBLEM:

GC of methyl esters is designed to separate and quantitate the individual compounds of methyl esters. The result is a chromatogram which can contain ten or more substances. To obtain accurate quantitative data each compound must be clearly separated from one another and also individually calibrated.

When the starting alcohol is a mixture rather than a pure form of methanol or ethanol a mixture of esters is formed during transesterification. Multiply the number of different types of esters in the original starting material by the number of different alcohols and now you get the total number of ester peaks in the chromatogram. If 10 esters make up the first sample of biodiesel made from pure methanol, then 20 make up the total with a mixture of methanol and ethanol. The greater complexity of the alcohol starting materials compounds the laboratory effort to quantify, and magnifies the likelihood of errors in calibration. Also, non esters are more likely to be interferences, with so many potentially quantifiable peaks.

NEED:

Research is needed to find ways to simplify the calibration in GC work while retaining the precision and accuracy and flexibility needed to do this work on a practical basis. The evaluation of methods for determining the total methyl esters using techniques which allow for a simplified approach to calibration, and greater selectivity for biodiesel esters. The new methods should strive to equal or exceed the total precision and accuracy available in existing methods, and, if requiring different instrumentation show promise for determining glyceride impurities in biodiesel.

2) PROBLEM:

Reference standards for the calibration of total methyl ester determinations introduce biases which are easily overlooked. For example, pure chemicals are often stated to be better than 99% pure, but the exact purity is not always stated, and the certification process can differ from company to company. Standard substances also have a shelf life, as do the prepared solutions, and the shelf life of the mixtures is unknown.

NEED

a) A rapid method for checking the purity of reference standards wouid greatly lessen the uncertainty associated with use of "pure" chemical standards. Develope specific, practical laboratory procedures for identifying the purity of and maintaining the integrity of standards used in the laboratory.

b) An evaluation of the conditions for storage and use of standard solutions and pure methyl ester standards is needed, with a recommended practice. Evaluate the stability of individual compounds, and identify prospective avenues for maintaining the standards at their certified initial purities. The shelf life of various standards needs to be established and the appropriate procedures for maintaining and recognizing standards and standard solutions at their certified values need to be identified.

c) The practices required to establish the exact purity of reference standards need to be evaluated and compared to what is practiced currently by organizations manufacturing and vendoring standards.

3)PROBLEM:

Internal standards are used in chromatographic analysis to adjust for variable injection volumes, wandering compound retention times, and quantitation. In GC especially, the internal standard can play a major role, and a suitable internal standard offers the potential for enhanced certainty associated with peak identification, and manual injection, thereby reducing instrument costs considerably. The application of odd number carbon fatty acid methyl esters is limited by the prevalence of naturally occurring low level odd number acid methyl esters, which can distort the quantification process. Internal standards can also pose a problem if their chromatographic properties differs substantially from those of the compounds of interest. There are currently no suitable internal standards.

NEED:

The concept of internal standardization as applied to the high precision and accuracy requirements of biodiesel needs study to evaluate its applicability to total methyl ester determinations. Evaluate and recommend candidate internal standards, and test experimentally in gas chromatographic determinations of total methyl esters.

4) PROBLEM:

Extension of GC methods to petroleum/biodiesel blends is complicated by interferences in ester determinations by petroleum hydrocarbons.

NEED:

Investigate ways to estend GC methodology to biodiesel blends, test and recommend the "best" possible adaptation.

5) PROBLEM:

The development of laboratory analytical technology for analyzing components in biodiesel has undergone considerable advances in the last ten years, particularly in HPLC detection technology. The implementation of gas chromatography capillary techniques to this application is well over ten years old, and still poses numerous challenges in quantitation.

NEED:

Investigate technologies associated with HPLC detection and its potential application to biodiesel glyceride determinations and total methyl ester determinations. Test and recommend the "best" possible technology.

DRAFT

WEIGHT PERCENT TOTAL METHYL ESTER CONTENT BY GC- 1/29/96

INTRODUCTION/SCOPE

This method is applicable to the determination of total methyl ester content of pure biodiesel fuels comprised of the methyl esters of fatty acids (FAME) having 8-24 carbon atoms. This includes methyl esters derived from animal fats, vegetable and marine oils. As such, the method permits quantitative separation of methyl esters containing saturated and unsaturated residues of fatty acids. This method fails to measure the ester content of polymerized substances or esterified osidation products of fatty acids.

This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

ADDITIONAL APPLICATIOYS

This method may also be used to determine the unsaturation content of FAME by an estimation of the iodine number through method AOCS Cd lc-85. The density (specific gravity), vapor pressure as a function of temperature, boiling point distribution, are among the possible properties which may be calculated for pure materials having the composition determined by this method.

SUMMARY

The biodiesel fuels are first pretreated by passage through a microcolumn dissolved in hexane, then analyzed by gas chromatography with flame ionization detection, using cool, on-column injection techniques. Autoinjection of external standards is used for calibration. The solutions are analyzed using a moderately polar megabore (0.53mm) capillary column.

Each individual ester is determined by comparison to standard solutions of pure ester compounds. Response factors are established for each compound and used to convert raw data into weight percent figures. The weight sum of the individual esters is the total methyl ester content.

REFERENCE

This method is a modification of AOCS standard method Ce-le-91 (22), to accommodate the requirements for determination of total methyl esters in biodiesel. Specifically, the following modifications are introduced for optimized precision and accuracy:

1) Only cool on column injectors with stable inlet temperatures on injection at less than 50 degrees C are specified (this is an option in the AOCS method).

2) A megabore (0.53 mm) column with 1.0 um film thickness is specified. Here, some resolution capability is sacrificed with a gain in sample capacity, decreased analysis times, and simplicity in sample and gas flow path. The temperature program and carrier flows are also modified to accommodate the megabore column.

3) Samples are pretreated to remove polar substances insoluble in hexane.

4) An autoinjector system is required to attain the necessary precision.

5) A computer/data system is necessary.

6) Helium is specified as carrier gas for safety reasons.

EQUIPMENT AND APPARATUS

1) A gas chromatograph capable of multiple temperature programming with direct, cool, on-column capillary injection and a flame ionization detector (FID). An autosampler is necessary.

2) Chromatography software/computer interface/with integrator capability, data automation capability preferred.

3) Polyethylene glycol bonded phase capillary column, 30 M x 0.53mm ID, 1.00 um film thickness or equivalent (better resolution, but lower sample capacity with thinner films), capable of baseline resolution of C18-0, C18-1, and C18-2 methyl esters @ 10:50 weight percent, and a resolution equal to or better than for the separation of C22:0, and C22:1.

4) A high precision 10 ul autoinjector syringe.

5) Analytical micro balance capable of 5 decimal (0.01mg) readings.

6) Cap sealed borosilicate volumetric flasks,, 10 and 100 ml.

7) Carrier gas purification system to remove oxygen and moisture traces to below 2 ppm.

CHEMICALS AND REAGENTS

Solvents:

All solvents used in this method are HPLC or GC high purity, low residue grade.

Standards

High purity >99% substances for standards, certified by the vendor with plus or minus tolerances. Mixtures of esters from the vendor should be certified for composition and weight content of methyl esters within acceptable tolerances. To minimize handling of the standards, the exact weight of esters in a given ampule, and the total weight should be specified.

Gases

1) Carrier and makeup gas- helium 99.995% minimum with moisture and oxygen removal capability to less than 2 ppm.

2) Hydrogen and air for FID, zero grade, or hydrogen, 99.9% minimum free of organic impurities and oxygen.

SAMPLE PREPARATION/CLEANUP

Approximately 100.00 mg of transesterified product is transferred directly to the inside of an RP CN disposable micro column (Waters CN Seppak was found to work). Wet the upper half of the microcolumn with pentane. Allow to stand 2 minutes. Then pass 10 ml of hexane through the column at a rate of about 10 ml/minute following the manufacturers recommendations, and into a 100 ml volumetric flask. Dilute to volume with hexane.

REPLICATION

At minimum samples and standards should be prepared in duplicate and each duplicate analyzed in duplicate.

GC COLUMN CONDITIONS

Carrier: He, 5.0 ml/min
Injector T: 50 c
Detector T: 250 C
Column Program:
40 C /0 min 15 C/min to 120/0 min 4 C/min to 250C/hold 2 min.

INJECTIONS

A 2 ul aliquot is injected rapidly, with full depression of the needle into the septum.

STANDARDS AND CALIBRATION

Standards are prepared from either certified standard mixtures with a stated composition within 10 percent relative of each component in the unknown sample, or by manual preparation of mixtures from pure, certified standard materials. The sample is first run with results compared to a prechosen standard mixture thought to resemble the sample. If the area of each component greater than 1% (of the total methyl ester area) is within %10 of the calibrated standard component, then the results calculated from the standard stand. If not, then each component outside of the 10 % range must be calibrated separately. Response factors for components less than 1.0% down to C12 are arbitrarily assigned a value of 1.00, compared to a C18 methyl ester standard and additional calibration of such components is unnecessary.

The pure standard material in milligrams should be weighed to the nearest 0.01 milligram, and quantitatively transferred to a 100 ml volumetric flask for analysis. The weight of commercially available standard mixtures should be certified for weight content, in addition to composition. For these mixtures complete direct transfer of the content with careful washings of solvent are recommended to avoid unnecessary handling and subsequent losses of slightly volatile materials.

CALCULATIONS

The mass of all individual components is summed to obtain the total methyl ester content. This is then divided by the total sample weight and multiplied by 100 to obtain a percentage total.

Obtain the area for each peak and express it as percent of the total fatty acid methyl ester.

The individual components are calculated from the data in the following way.

Area (SAMPLE COMPONENT)/Area (STD)=
Mass(SAMPLE COMPONENT)/mass(STANDARD)

or

$$\text{Mass(SAMPLE COMPONENT)} = \frac{\text{Area (SAMPLE COMPONENT) X mass (STANDARD)}}{\text{Area (STANDARD)}}$$

Total methyl ester percent (TEP)=

$$\text{TEP} = 100 \text{ X Sum of masses/ Total sample weight}$$

ACCURACY AND PRECISION

The precision of the method is evaluated by a series of replicate injections. The precision is calculated as the square root of the sum of the variances associated with each component. A total precision of plus or minus 0.5% relative is attainable for total methyl ester determinations using this method.

The accuracy of the method is unknown and depends on at least three factors. The first is the accuracy of certified reference standards, and the biases introduced by laboratory mixture preparation and handling. The second is errors in integration, e.g. those introduced by unresolved peaks, shoulders, etc. The third is interferences.

Standards certified on the basis of purity and weight are available from several commercial sources. Certification tolerances for the standards are unspecified at the moment, but consideration of the total bias introduced by these tolerances must be given, and clearly should be at a minimum within acceptable limits with consideration of the limits of total ester content of biodiesel specifications.

QUALITY CONTROL/QUALITY ASSURANCE

At minimum, samples and associated standards should be analyzed in duplicate. A quality program should be established which reflects an on-going monitoring of the precision of the method and subtle changes in the analysis system, and which assists in identifying the degree of replication required to validate biodiesel specifications.

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