

Evaluation of Low Concentrations of Steryl Glucosides in Biodiesel by Gas Chromatography

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Publication Date: January 25, 2010

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Please cite this article in press as White, J.M., et al., "Evaluation of Low Concentrations of Steryl Glucosides in Biodiesel by Gas Chromatography," Renewable Energy Group, Inc., www.regfuel.com (2010)

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1 Executive Summary

This report discusses the measurement of steryl glucosides (SG) in biodiesel at low levels using a Gas Chromatography-Flame Ionization Detector (GC-FID). SG are minor constituents in some biodiesels that have been associated with the formation of sediments above the cloud point. Currently available GC-FID procedures do not detect SG below 10 ppm in biodiesel.

A procedure was created to detect SG directly in biodiesel. This procedure uses a GC-FID method that measures SG in biodiesel down to 2.5 ppm with a standard deviation of ± 1.7 ppm.

While developing the GC-FID procedure, matrix effects were detected in the silylation step. It is recommended that when measuring SG in a new matrix, a sample spiked with a known amount of SG should be run to check for the presence of matrix effects. To minimize matrix effects, an investigation into the silylation reaction conditions was done. The optimum reduction in matrix effects was found at 2.00 mL pyridine, 0.30 μ L internal standard, 1.0 mL *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and 30 minutes at 60 °C.

To extend the accuracy of the GC-FID to lower levels of SG, solid phase extraction (SPE) was used to concentrate SG present in biodiesel before measurement by GC-FID. SPE combined with GC-FID was shown to detect SG in distilled biodiesel down to 0.5 ppm with a standard deviation of ± 0.53 ppm. The SPE combined with GC-FID on undistilled biodiesel was found to be ineffective as undistilled biodiesel may have contained some components which interfered with the absorption of SG during the SPE step.

2 Acknowledgments

This report was prepared with the support of the Iowa Power Fund Board and the Office of Energy Independence. However, any opinions, findings, conclusions or recommendations expressed herein are those of the authors and do not necessarily reflect the views of the Iowa Power Fund Board of the Office of Energy Independence.

3 Disclaimer

This report contains guidelines, procedure and protocols for performing reactions and testing that includes biodiesel, petro diesel, specialty chemicals and solvents. The authors in no way imply that these procedures are described in complete details or are safe to reproduce. When performing chemical testing or analyzing products, there is no substitute for good judgment and thorough background research on hazards and toxicities.

A list of possible hazards and hazardous environments when synthesizing and testing products described in the report include, but are not limited to: mechanical failure; high pressures; high temperature; high voltage, chemical toxicity, chemical reactivity, chemical explosion, acid burns and toxic vapors

The authors assume no responsibility for any incident that occurs when reproducing procedures similar to or the same as described in this report.

4 Introduction

Steryl glucosides (SG) are among the components that have been observed in minor quantities in some biodiesels.^{1, 2, 3} SG have been implicated in filter plugging and have been associated with the formation of precipitates in biodiesel above the cloud point.^{4, 5} Tang et al. have reported SG as sediments,³ and Lee has addressed that SG may be involved in the precipitation of monoglycerides.⁶ Pfalzgraf has observed that approximately 10 ppm SG may cause long cold soak filter times.⁴

Currently there are a few procedures that can measure SG in biodiesel such as gas chromatography using flame ionization detector (GC-FID), high pressure liquid chromatography (HPLC), and gas chromatography/mass spectrometry (GC/MS). GC-FID procedures can measure SG in the 10-20 ppm range in biodiesel.^{1, 3} Also, the current GC-FID procedures require a separate sample preparation step. One GC-FID procedure prepares the sample by centrifugation and filtration.³ Another technique steam distills the biodiesel and then measures SG in the residue by GC-FID.¹ The same author has also described a GC-FID procedure to analyze SG from solid residues after a multiple step extraction involving five solvents.¹ In addition to GC-FID, there are HPLC and GC/MS procedures in the literature that can measure SG in biodiesel down to 30 ppm and 5 ppm, respectively.^{2, 7, 8}

A GC-FID procedure that could detect SG below 10 ppm accurately in biodiesel will be beneficial to the industry. GC-FID procedures may be readily transferred to biodiesel facilities since the ASTM D6751 standard for biodiesel uses GC-FID for testing free and total glycerin (ASTM D6584).⁹ The GC column for the GC-FID procedure is the same column used for ASTM D6584 so the column would not have to be changed out to run both procedures. The silylation chemicals are common to biodiesel plant laboratories and the procedure may be easily incorporated into quality control testing. Laboratories that are performing free and total glycerin should be able to run the GC-FID procedure at a comparable cost.

In this report, a GC-FID procedure capable of quantifying SG below 10 ppm in biodiesels is demonstrated. The development of an improved GC-FID sample preparation to minimize matrix effects is described. Also presented is the use of solid phase extraction (SPE) as a novel way to concentrate SG before measurement by GC-FID.

5 Direct Measurement of SG in Biodiesel by GC-FID

For SG to be measured by GC-FID they must be reacted with a silylating agent. This silylation makes the SG more volatile so it can be separated from the biodiesel in the GC column. The direct measurement of SG involves the injection of the silylated biodiesel mixture straight onto the GC-FID. This procedure does not involve a SG concentration step like other methods in the literature making the direct measurement a simpler process.^{1, 3} To detect lower amounts of SG by direct measurement the silylated sample size of the biodiesel was 0.5000 g. The biodiesel concentration in the sample

preparation was further increased by the elimination of the heptane dilution step used in other GC-FID methods.^{1, 3} The following procedure describes the quantification of SG on the GC-FID via injection of a silylated biodiesel sample.

5.1 Materials and Methods

Materials

The undistilled biodiesel utilized in this study was obtained from a commercially available source. The material was distilled using an 18 inch Vigreux column under vacuum < 1 torr, and to a maximum temperature of 210 °C. The distillate fraction was collected between 86 and 187 °C.

The SG standard was prepared in the laboratory from a crude sample obtained from a biodiesel production plant. The crude SG was dispersed in about 10 volumes of hot (45-60 °C) methanol, filtered, washed with hot methanol, and dried. This procedure was repeated 3 times to remove minor components. A 150 ppm solution of the washed SG in heptane was prepared and analyzed on a GC-FID calibrated with a previous SG sample of known purity and concentration. The measured concentration was compared against the prepared concentration to confirm the SG concentration. The GC-FID chromatogram was also examined for minor components especially in the sterol region and the acylated steryl glucosides region. The solid sample of washed SG was analyzed on a Fourier Transform Infrared Spectroscopy (FTIR) instrument to check for other materials especially in the soap region (1580 - 1530 cm^{-1}). If other components were detected or if the measured concentration was not within experimental error of the prepared concentration, another hot methanol wash was performed and the material was retested by GC-FID and/or FTIR.

The heptane (HPLC grade) was purchased from Fisher Scientific Inc. The pyridine was obtained from Sigma-Aldrich Co, grade ReagentPlus[®]. The 1,2,4-butanetriol and tricaprln used were Supelco (Sigma-Aldrich Co.) Internal Standard #1 and #2 respectively. The *N,O*-bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane (BSTFA with 1% TMCS) was purchased from Pierce Biotechnology Inc. (Thermo Fisher Scientific, Inc.). All reagents were used as received unless otherwise stated.

FTIR Analysis

The purity of laboratory made reference materials was investigated with a PerkinElmer 100 spectrometer with attenuated total reflectance (ATR) sampling attachment and a resolution of 4° per cm^{-1} . The spectra were taken at room temperature and in a range of 4000 – 650 cm^{-1} . In all cases, air spectrum was used as background.

GC-FID Analysis

Apparatus

A PerkinElmer Clarus 600 GC-FID equipped with a Restek MXT Biodiesel TG 15 m, 0.32 mm ID, 0.10 μm column with a 2 m, 0.53 mm guard column was used to quantify SG.

Table 1 GC-FID operating conditions

Injector		
Cool on column injection		
Sample size	1 μ L	
Column Temperature Program		
Initial temperature	80 °C	
Rate 1	15 °C/min to 305 °C	
Rate 2	5 °C/min to 335 °C	Hold 5 min
Rate 3	15 °C/min to 365 °C	Hold 5 min
Detector		
Type	Flame ionization	
Temperature	380 °C	
Range	1	
Attenuation	-6	
Carrier Gas		
Type	Hydrogen or helium	Measured at 50 °C
Flow rate	3 mL/min	

Sample Preparation 1*

Prior to injection, the sample was prepared by dissolving 0.5000 g in 2.00 mL of pyridine, to which was added 30 μ L of internal standard. The internal standard was prepared as specified in ASTM D6584 using butanetriol and tricaprin.⁹ To this mixture was added 1.0 mL of BSTFA with 1% TMCS. The mix was well shaken and heated at 60 °C for 30 minutes.

Sample Preparation 2**

Prior to injection, the sample was prepared by dissolving 0.5000 g in 1.00 mL of pyridine, to which was added 30 μ L of 1,2,4 butanetriol and tricaprin as internal standard. The internal standard was prepared as specified in ASTM D6584.⁹ To this mixture was added 1.0 mL of BSTFA with 1% TMS. The mix was well shaken and allowed to stand for at least 30 minutes.

Calibration

The calibration curve (0 to 22 μ g) was constructed using samples of distilled soy biodiesel spiked with known amounts of SG. The calibration curve was calculated on Stat-Ease, Design-Expert Version 7.1.1 using the mass of SG versus the SG response ratio from the GC-FID.

*Recommended sample preparation conditions from section 5.3 Matrix Effects on Silylation

**Sample preparation conditions are those used in sections 5.2 and 6.2.

5.2 Results and Discussion

Before the GC-FID can be used to measure SG in biodiesel, a calibration curve must be determined. For the direct measurement of SG, the GC-FID was calibrated with SG standards in distilled soy methyl ester. The distilled soy methyl ester was analyzed by GC-FID for SG and no SG peaks were found, confirming that no detectable SG was present in the distilled soy methyl ester (see Fig. 1). The calibration curve was calculated over a range of 0 to 22 μg SG (0-40 ppm) using the mass of SG versus the SG response ratio from the GC-FID. The resulting calibration curve had an R^2 of 0.9919 and the lower limit of detection was determined to be 2.5 ppm which was based on three times the noise in the SG region of the chromatogram. The standard deviation for SG measured in the biodiesel was ± 1.7 ppm. The calibration data is presented in Fig. 2.

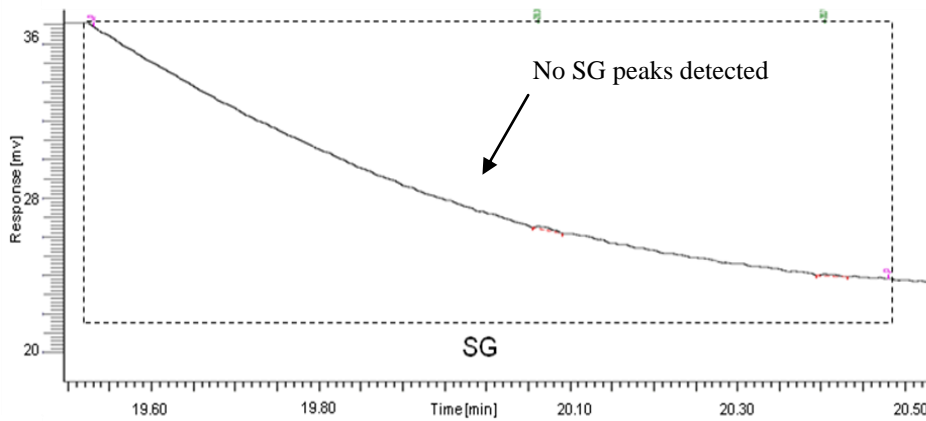


Fig. 1 GC chromatogram of the SG region of distilled soy methyl esters

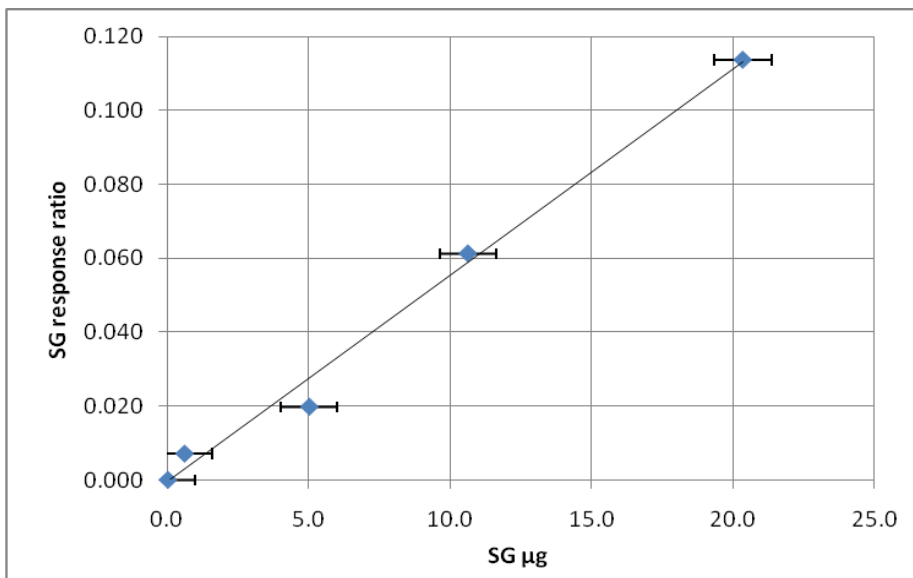


Fig. 2 GC-FID calibration data

The calibration curve was checked by running 4 samples at different SG concentrations; the results are shown in Table 2. The measured values (determined using the calibration curve) were similar to the actual values (determined by the sample preparation). The agreement of the actual values and the measured values supports that this calibration curve can be successfully used to measure SG in biodiesel down to 2.5 ppm with a standard deviation of ± 1.7 ppm. A typical GC-FID chromatogram of distilled soy methyl esters with SG is shown in Fig. 3. The SG peaks were found in the region between 19.5 and 21.0 minutes.

Table 2 Calibration check samples analyzed directly by GC-FID

SG actual (ppm)	SG measured (ppm)
5.0	3.5
9.8	7.9
20.8	22.3
40.1	40.6

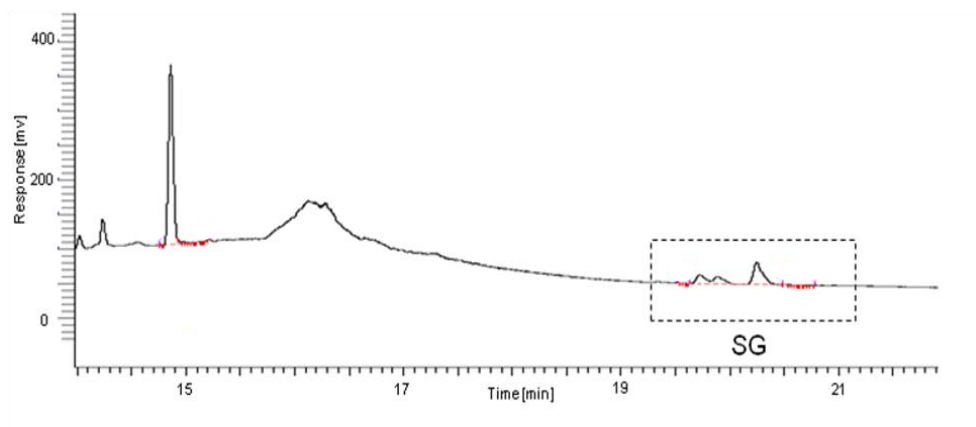


Fig. 3 GC-FID chromatogram of distilled soy methyl ester with SG showing internal standard tricaprins at retention time 14.9 min. and SG at retention time 19.5-21.0 min.

5.3 Matrix Effects on Silylation

For the GC-FID procedure developed in this study, the measurement of SG was found to be sensitive to the matrix in which the SG was present. The graph in Fig. 4 illustrates the matrix effect which shows the SG response ratio falling along 2 separate lines indicating that the same amount of SG was yielding different SG response ratios depending on the matrix. The sensitivity of the SG measurement to the matrix could be due to the silylation step in the GC-FID procedure since the silylation reaction with BSTFA is known to be subject to matrix effects.¹⁰

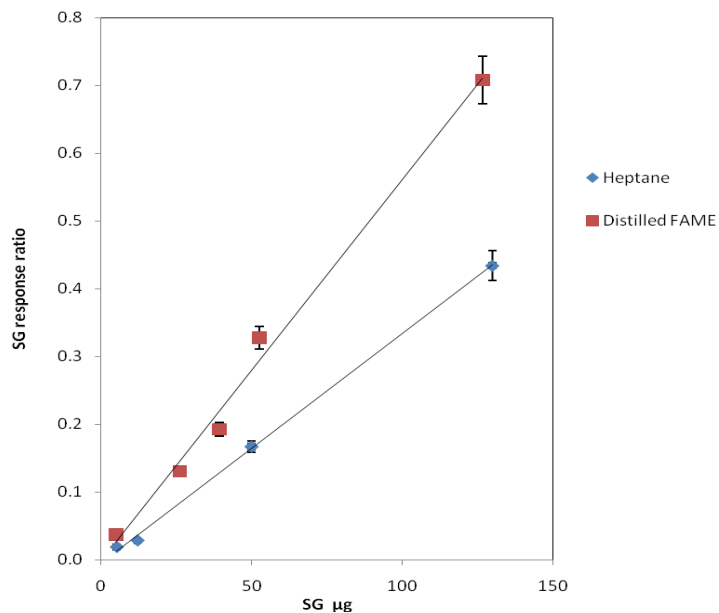


Fig. 4 Matrix effects on SG calibration

The observed matrix effects may be minimized if the silylation reaction goes further to completion. Therefore, a screening design was undertaken to determine the effects that the reaction conditions had on the baseline silylation reaction (section 5.1 GC-FID Analysis, Sample Preparation 2). The procedure used was a 3 factor 2 level factorial design. The factors were the amount of BSTFA (1.0 versus 2.0 mL), the amount of pyridine (1.0 versus 2.0 mL) and reaction temperature (23 versus 60 °C). Experiment 1 in Table 3 was the current GC-FID sample preparation and was included as the baseline reaction conditions. For each sample preparation, the following conditions were kept constant; 0.5000 g of distilled soy biodiesel spiked with 101.2 ppm SG, 30 µL of internal standard and the mixture was allowed to stand for 30 minutes at the reaction temperature.

Table 3 Reaction condition effects on silylation

Reaction	Conditions			
Experiment	BSTFA mL	Pyridine mL	Temperature °C	R/R ₁
1	1.0	1.0	23	1.00
2	1.0	1.0	60	1.08
3	2.0	1.0	23	1.02
4	2.0	1.0	60	0.99
5	1.0	2.0	23	0.93
6	1.0	2.0	60	1.40
7	2.0	2.0	23	1.28
8	2.0	2.0	60	1.13

Since the SG response ratio is related to the silylated SG concentration,⁹ the improvement in the silylation reaction was determined by the SG response ratio for the given reaction conditions (R) divided by SG response ratio for the baseline reaction conditions (R₁). Therefore, the higher the R/R₁ value, the more the reaction is driven towards completion relative to the baseline conditions.

The largest R/R₁ in Table 3 corresponds to the reaction conditions of 1.0 mL BSTFA, 2.0 mL pyridine, and 60 °C (experiment 6). The silylation at these reaction conditions went further to completion relative to the baseline conditions than the other trials which may indicate less interference with matrix effects. Hence, it is recommended that the silylation reaction conditions for the GC-FID should be modified to 1.0 mL BSTFA, 2.0 mL pyridine, and 60 °C (experiment 6).

6 SPE in Combination with GC-FID

In order to measure SG in biodiesel to lower levels than the GC-FID can detect alone, SPE was used to concentrate SG from the biodiesel before quantification on the GC-FID.¹¹ The SPE procedure uses a SPE cartridge to separate the SG from the biodiesel, solvent washes to remove the SG from the SPE cartridge, and the removal of the solvent to concentrate the SG. After SG has been concentrated, the SG in the sample is derivatized with BSTFA and analyzed by GC-FID. The following section describes the development and demonstration of an SPE procedure to separate SG in distilled biodiesel.

6.1 Materials and Methods

Materials

The heptane (HPLC grade), methanol, tetrahydrofuran (THF), chloroform, ethyl acetate, and dichloromethane were purchased from Fisher Scientific Inc. The pyridine was obtained from Sigma-Aldrich Co, grade ReagentPlus[®]. The 1,2,4-butanetriol and tricaprin used were Supelco (Sigma-Aldrich Co.) Internal Standard #1 and #2 respectively. The *N,O*-bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane was purchased from Pierce Biotechnology Inc. (Thermo Fisher Scientific, Inc.). All reagents were used as received unless otherwise stated.

Cartridge Screening for SPE

SPE cartridges from Alltech were used to determine the type and size of the absorbent bed necessary to absorb SG as well as the dosage of the biodiesel sample. The cartridges were conditioned with 3-5 g of heptane depending on the size of the absorbent bed. The heptane was added to the cartridge and allowed to drain by gravity. Distilled soy biodiesel of known SG concentration was added to the cartridge and drained at the rate of 1 drop every 1-3 seconds. The rate of drain was controlled by applying air pressure to the top of the cartridge barrel. The eluate was collected in a different tared scintillation vial and the heptane was removed on a hotplate at 130 °C. The eluate was analyzed by GC-FID. The chromatogram was examined in the SG region to determine if the SG peaks were more than the background. If no SG peaks were detected, the eluate was passed through another 200 mg silica bed Alltech cartridge conditioned with 3 g of heptane. The eluate was drained at a rate of 1 drop every 1-3 seconds and collected in a separate tared scintillation vial. The cartridge was washed twice with 2.0 g of 4:1 by weight of methylene chloride/methanol solution. The washes were drained from the cartridge by gravity, were collected in another tared scintillation vial and the methylene chloride/methanol was removed on a hotplate at 130 °C. After the removal of the methylene/chloride, the weight of the contents in the scintillation vial was brought to 0.5000 g by the addition of heptane. The whole sample was analyzed using GC-FID. The chromatograms were examined in the SG region to determine if SG could be detected.

Solvent Screening for SPE

Different solvents were screened to determine the best candidate to wash SG from the cartridges. A silica bed Alltech cartridge was conditioned with 3-5 g of heptane. The heptane was added to the cartridge and allowed to drain by gravity. A distilled biodiesel of known SG concentration was added to the cartridge and drained at the rate of 1 drop every 1-3 seconds. The rate of drain was controlled by applying air pressure to the top of the cartridge barrel. The eluate was collected in a tared scintillation vial. The solvent washes were added to the cartridge and were drained by gravity. The solvent washes were collected in a different tared scintillation vial and the solvent was removed on a hotplate at 130 °C. The weight of the contents after solvent removal was brought to 0.5000 g by the addition of heptane. The entire sample was analyzed using GC-FID.

SPE Procedure for Determination of SG in Biodiesel

A 200 mg silica bed Alltech cartridge was conditioned with 3 g of heptane. The heptane was added to the cartridge and was allowed to drain by gravity. A 10.0000 g sample was added to the cartridge and drained at the rate of 1 drop every 1-3 seconds. The rate of drain was controlled by applying air pressure to the top of the cartridge barrel. The eluate was collected in a tared scintillation vial. The cartridge was washed twice with 2.0 g of 4:1 by weight of methylene chloride/methanol solution. The washes were drained from the cartridge by gravity. The washes were collected in a different tared scintillation vial and the solvent was removed on a hotplate at 130 °C. The weight of the contents after solvent removal was brought to 0.5000 g by the addition of heptane. The whole sample was analyzed on the GC-FID and the SG concentration was determined based on the original amount of sample put on the SPE cartridge

GC-FID Analysis

The GC-FID was performed as per section 5.1 GC-FID Analysis.

6.2 Results and Discussion

In the development of an SPE procedure, the following variables need to be determined: the amount of sample loaded on the cartridge, the type and size of cartridge to absorb the SG, as well as the type and amount of solvent to wash the SG from the cartridge. To test the viability of the developed SPE procedure, the SPE method was used to concentrate the SG in distilled and undistilled biodiesel before injection onto the GC-FID (see section 5.1 GC-FID Analysis, Sample Preparation 2).

Screening of SPE Cartridges

To determine the best SPE cartridge for the removal of SG from distilled biodiesel, 3 separate cartridges were evaluated. For each cartridge a 3-level partial factorial design was used to assess the performance of each cartridge in regards to biodiesel sample size, wash solvent, and the amount of wash solvent. The biodiesel sample size varied from 5 to 10 g. These amounts were chosen because they give a concentration increase of 10 to 20 times for the SG. For the wash solvent, heptane and isopropanol were chosen because they gave a wide range of solvent polarity. The amount of wash solvent was varied from 2 to 10 g. After each extraction, the SG in the eluate, SG in the wash, and total recovery of biodiesel were measured. These values are expressed in percent recovery. The design of the experiment and the results are summarized in Table 4.

Table 4 Screening of SPE cartridges

SPE separation conditions								
SPE bed	Bed size (mg)	Biodiesel sample (g)	SG conc. (ppm)	Wash solvent	Wash solvent (g)	SG in eluate (% recovery)	SG in wash (% recovery)	Total biodiesel recovered (% recovery)
Alumina N	500	10	21.8	heptane	2	ND	ND	98.71
Alumina N	500	5	21.8	heptane	10	ND	ND	98.86
Alumina N	500	5	21.8	isopropanol	2	ND	ND	99.50
Alumina N	500	10	21.8	isopropanol	10	ND	ND	98.41
Silica	1000	10	21.8	isopropanol	10	ND	45.0	99.71
Silica	1000	10	21.8	heptane	2	ND	ND	96.89
Silica	1000	5	21.8	isopropanol	2	ND	43.9	98.54
Silica	1000	5	21.8	heptane	10	ND	ND	96.76
Silica	200	5	21.8	heptane	10	ND	ND	99.47
Silica	200	5	21.8	isopropanol	2	ND	25.8	98.67
Silica	200	10	21.8	heptane	2	ND	ND	98.67
Silica	200	10	21.8	isopropanol	10	ND	81.8	99.09

ND = none detected

No SG were detected in any of the eluates from both types of cartridges, showing both neutral alumina and silica were good candidates for removal of SG from biodiesel. For the alumina bed, the wash data for heptane and isopropanol showed no SG, therefore the alumina bed was not chosen for further studies.

The 200 mg silica bed was chosen over the 1000 mg silica bed since more SG were removed by the washes from the 200 mg bed than the 1000 mg bed. To screen the performance of the different solvents for removing SG from the SPE cartridges, the 200 mg silica bed was used.

Screening of Wash Solvents

Once the SG have been caught by the absorbent, they must be removed for quantification. A variety of solvents were tested as possible candidates to remove SG from the silica bed. The solvents examined were pyridine, tetrahydrofuran (THF), chloroform, isopropanol and 4:1 methylene chloride/methanol (4:1 CH₂Cl₂/CH₃OH). These solvents were chosen because they have been reported to dissolve SG or have been used in previous chromatographic processes and are compatible with the technique.^{1, 2, 10} The effectiveness of the washes to remove SG was examined at the 2 and 4 g addition levels. The results of the screening experiments are summarized in Table 5.

The effectiveness of SG removal from best to worst was 4:1 methylene chloride/methanol > THF = pyridine > isopropanol > chloroform. Pyridine was not chosen due to health hazards, foul odor, and because it was more difficult to evaporate. From the rest of solvents tested, only the 4:1 methylene chloride/methanol washes presented a good combination of advantages and performance. It presented the high

percent recovery of SG from the silica bed and it was also easy to remove on a hotplate at 130 °C.

A sample size of 10 g was selected for the procedure since it loaded more SG onto the cartridge than 5 g. This made the preferred SPE conditions for the separation of SG as a 10.00 g sample size, a 200 mg silica cartridge, and two 2.0 g washes of 4:1 methylene chloride/methanol. The detailed SPE separation procedure is in section 6.1 SPE Procedure for Determination of SG in Biodiesel.

Table 5 Screening of wash solvents

SPE separation conditions								
SPE bed	Bed size (mg)	Biodiesel sample (g)	SG conc. (ppm)	Wash solvent	Wash solvent (g)	SG in eluate (% recovery)	SG in wash (% recovery)	Total Biodiesel recovered (% recovery)
Silica	200	5	21.8	THF	4	--	96.7	99.7
Silica	200	5	21.8	Pyridine	2	ND	88.3	103.0
Silica	200	5	21.8	Chloroform	4	--	1.5	98.4
Silica	200	5	21.8	Pyridine	4	ND	167.5 *	129.6 **
Silica	200	5	21.8	Isopropanol	4	--	56.8	99.5
Silica	200	5	21.8	Chloroform	2	ND	1.6	100.4
Silica	200	5	21.8	THF	2	--	89.2	99.2
Silica	200	5	21.8	Isopropanol	2	ND	43.1	100.3
Silica	200	5	21.8	4:1 CH ₂ Cl ₂ /CH ₃ OH	2	--	102.0	--
Silica	200	5	21.8	4:1 CH ₂ Cl ₂ /CH ₃ OH	4***	--	113.0	--
Silica	200	10	3.5	4:1 CH ₂ Cl ₂ /CH ₃ OH	4***	--	103.0	--

ND = none detected

*GC has significantly lower counts for the tricaprln internal standard

**incomplete pyridine removal is suspected

***done as two 2 g washes

--analysis not performed

Evaluation of SPE for Determination of SG in Distilled Soy Biodiesel

The SG content was determined in a series of distilled soy biodiesel spiked with known amounts of SG. The SPE procedure (section 6.1 SPE Procedure for Determination of SG in Biodiesel) was used to concentrate the SG before injection into the GC-FID. The results of these runs are summarized in Table 6.

Table 6 Evaluation of SPE method at 5.0, 20.8 and 40.1 ppm SG

SPE separation conditions							
SPE bed	Bed size (mg)	Biodiesel sample (g)	SG conc. (ppm)	SG in eluate	Biodiesel in eluate (% recovery)	SG in wash (% recovery)	Total biodiesel recovered (% recovery)
Silica	200	10.00	5.0	ND	95.89	165.3	99.4
Silica	200	10.64	5.0	ND	95.84	167.1	99.8
Silica	200	9.98	20.8	ND	95.53	168.9	99.8
Silica	200	10.04	20.8	ND	95.95	177.0	99.9
Silica	200	9.94	40.1	ND	96.13	176.0	100.0

The total biodiesel recovery in every case was > 99 %, demonstrating that most of the mass of biodiesel was accounted for during the SPE treatment.

However, the 165 - 177 % recovery of SG revealed an underlying experimental problem not detected before in the preliminary analyses. In the previous experiment, the calibration curve was built using standards with distilled soy biodiesel as the matrix. The final SPE sample was a concentrate of minor components in distilled soy biodiesel, a different matrix than the samples used in the calibration set. It was suspected but the authors did not investigate that this difference in the sample matrix could have enhanced the reactivity of the SPE treated samples towards BSTFA.¹²

From Table 6, it was observed that the percent recovery of SG after the SPE treatment was fairly constant over the range of concentrations indicating a consistent variation in the data which could be described by means of another calibration equation. Therefore, a new calibration curve was constructed using SG standards that have gone through the SPE procedure. The calibration curve was calculated using SG standards from 2.5 ppm to 20.7 ppm. The new calibration curve had an R^2 of 0.9987 and the lower limit of detection was determined to be 0.5 ppm which was based on three times the noise in the SG region of the chromatogram. The standard deviation for SG in biodiesel was \pm 0.53 ppm.

The calibration was checked by running two samples at different SG concentrations, the results are shown in Table 7. The measured values were within the standard deviation (\pm 0.53 ppm) of the actual values. The close agreement of the actual values and the measured values supports that this calibration curve may be used to measure SG in biodiesel down to 0.5 ppm with a standard deviation of \pm 0.53 ppm.

Table 7 Calibration check samples analyzed by SPE/GC-FID

Check Samples	SG	SG
	actual	measured
5 ppm	5	4.6
2.5 ppm	2.5	2.7

Evaluation of SPE for Determination of SG in Undistilled Biodiesel

To determine the effects undistilled biodiesel may have on the SPE procedure, corn and soy biodiesel samples were chosen. The undistilled corn biodiesel was picked because the corn oil had a greater loading of minor components. The soy biodiesel was selected because soy oil is a dominant feedstock in the market and has a lesser amount of minor components.

Two problems developed when undistilled biodiesels were used in SPE. The first was an interference with the tricaprins peak used as internal standard. This interference in the undistilled corn biodiesel makes the chromatogram not viable to quantify SG due to presence of too many peaks in the region of interest (Fig. 5a).

To see if the interferences with the tricaprins peak could be removed, a series of washes varying in polarity were performed. In separate experiments, undistilled corn biodiesel was washed with 100% heptane, 25 % by weight ethyl acetate in heptane, and 50 % by weight ethyl acetate in heptane. The best solvent to remove the interferences with the tricaprins peak was pure heptane. The tricaprins region of the chromatograms for the different washes is shown in Fig. 5. The heptane prewash chromatogram in part (d) of Fig. 5 presents less overlapping of peaks than the other three chromatograms. A prewash step with heptane before the 4:1 methylene chloride/methanol wash should minimize interferences in the tricaprins region of the chromatograms.

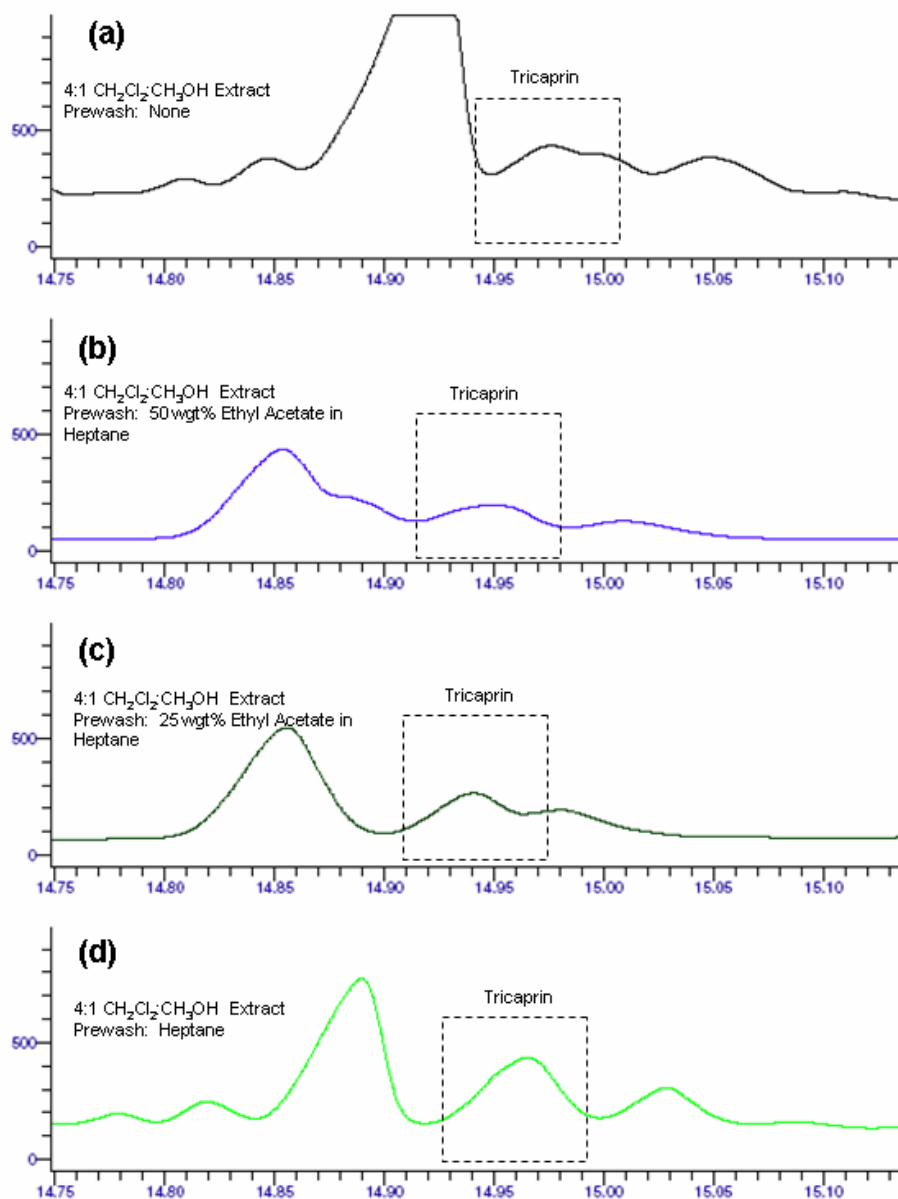


Fig. 5 Prewash effects on tricaprins peak in 4:1 CH₂Cl₂/CH₃OH extracts of undistilled corn biodiesel

The second difficulty was the SG in the undistilled corn and undistilled soy biodiesel were not being trapped on the cartridge. SG were observed in the chromatograms for both undistilled corn and undistilled soy biodiesel eluates. Typical chromatograms of these biodiesel eluates are shown in Fig. 6.

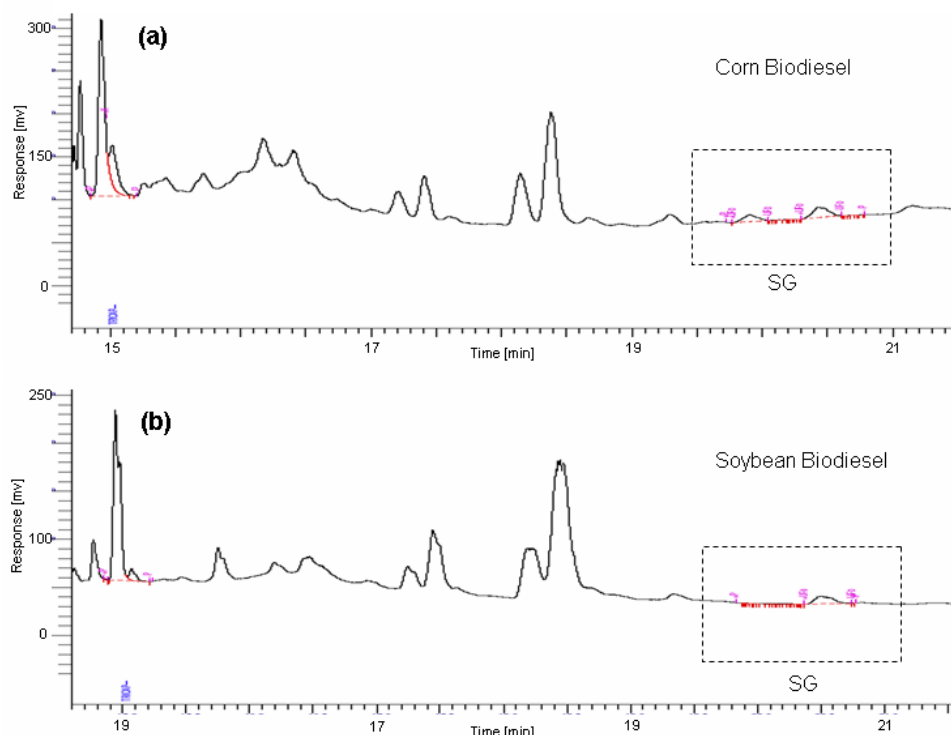


Fig. 6 SG in eluates of (a) undistilled corn and (b) undistilled soybean biodiesel

To determine SPE conditions that would completely absorb SG in undistilled biodiesel, a series of experiments, which varied biodiesel dose and silica bed size were performed. The results are summarized in Table 8. In the undistilled corn biodiesel eluates, the SG varied from 64-78 ppm with no obvious trend with biodiesel dose or silica bed size. Of the trials in Table 8, none was able to reduce the SG content of the eluates to the near zero levels required.

Table 8 SG in undistilled biodiesel eluates

Sample	Samples size (g)	Silica bed (mg)	Initial SG (ppm)	SG in eluate (ppm)*
corn biodiesel	10.0	200	84	78
corn biodiesel	4.0	200	84	67
corn biodiesel	4.0	200	84	74
corn biodiesel	2.5	200	84	71
corn biodiesel	5.0	1000	84	64
corn biodiesel	5.0	2000	84	78

*Calibration curve constructed with biodiesel standards without SPE pretreatment, the values are expected to be high

The lack of a decrease in the SG concentration in the undistilled corn eluates with changes in sample size, and bed weight could be due to the presence of other minor components in the undistilled biodiesel that interfere with the absorption of SG. These interferences imply that SPE may be unsuitable for the concentration of SG from undistilled biodiesel.

7 **Conclusions**

A GC-FID procedure that measured SG directly in biodiesel down to 2.5 ppm with a standard deviation of ± 1.7 ppm was developed.

During development of the procedure, the BSTFA silylation conditions used in the study were found to be affected by the matrix. Therefore, it is recommended that when unknown biodiesel samples are to be measured directly by GC-FID, samples spiked with SG should also be evaluated. The amount of the spiked SG recovered can be used to determine the extent of the matrix effect on the silylation reaction.

Further optimization of the silylation conditions to achieve less sensitivity to matrix effects showed that 2.00 mL pyridine, 0.30 μ L internal standard, 1.0 mL BSTFA and a reaction time of 30 minutes at 60 °C gave the best results. These sample preparation conditions were incorporated into the direct measurement of SG by GC-FID procedure.

An SPE in combination GC-FID procedure has been successfully used to measure low levels of SG in distilled biodiesel accurately. The SPE procedure can detect SG in distilled biodiesel down to 0.5 ppm with a standard deviation ± 0.53 ppm. The SPE combined with GC-FID was found to be incompatible with undistilled biodiesel as it contained components which interfered with the absorption of SG on the SPE cartridge.

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