Improving Biodiesel Monoglyceride Determination by ASTM Method D6584-17

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- ASTM D6584-17 was used to quantify monoglycerides in biodiesel from novel feedstocks
- The method was modified to include retention time matching for monoglycerides to better identify the correct peaks
- Results show improvement in monoglyceride determination with the modified method over the method as written

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ABSTRACT: Biodiesels produced from commercial and non-traditional feedstocks were analyzed by ASTM D6584-17 for monoglyceride (monoacylglycerol, or MG) content. It was found that D6584-17 as currently written may not accurately determine MGs from conventional feedstocks due to significant differences in retention time using modern instrumentation. For biodiesel from non-traditional feedstocks, D6584-17 did not sufficiently account for MGs containing fatty acids outside of C16 and C18 species. This

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1 led to under- and over-reporting of MGs, as critical components were not accurately measured.
2 Improvements to the method were made through a three-step process. First, a standard mixture of MGs was
3 run to determine the retention time of individual MGs that could be present in the samples from C10 to
4 C24. An additional analysis for the fatty acid methyl ester (FAME) profile was used to determine the major
5 MG species present in the biodiesel samples, using the assumption that the MG profile was proportional to
6 the FAME profile. The biodiesel samples were analyzed by D6584-17, and the MGs were identified using
7 retention time matching, based on the major species expected from measuring the FAME profile. By
8 combining these two methods, the accuracy of MG determination by D6584-17 was improved for biodiesels
9 prepared from all feedstocks.

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10 ***Keywords****: alternative feedstock; ASTM D6584-17; biodiesel; fuel quality; gas chromatography;*
11 *monoglycerides*

12 **1. INTRODUCTION**

13 Biodiesel (B100) is a renewable resource that is typically blended with petroleum diesel fuel before being
14 used in compression ignition engines, although it can be used as a neat fuel. ASTM International (ASTM)
15 defines biodiesel in Specification D6751-15ce1 as long-chain fatty acid monoalkyl esters [1]. ASTM
16 specifications are often utilized to enforce fuel quality and promote commerce and have been adopted by
17 many areas, including the United States and in many countries around the world.

18 Details of biodiesel production technology and methodology are reported elsewhere [2], [3]. The most
19 common feedstocks for B100 in the United States are soybean oil and other (or mixed) feedstocks [4], [5].
20 The term mixed feedstocks is used to describe mixtures of fats and oils and may include soybean, canola,
21 corn, and palm oils and rendered animal fats. These commodity fats and oils consist primarily of fatty acids
22 with 16 and 18 carbons (C16 and C18) and may contain up to three *cis*-double bonds. The ratios of the C16
23 and C18 fatty acids are unique for each feedstock and may be used to fingerprint feedstock sources.

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1 The Renewable Fuel Standard mandates the use of biodiesel and other alternative fuels in the United States.
2 The mandated volume of biomass-based diesel in 2018 and 2019 is 2.1 billion gallons [6]. The U.S. Energy
3 Information Administration reported over 1.5 billion gallons of biodiesel were produced in the United States
4 in 2017, the last complete year available [7]. One requirement of this mandate is that biodiesel must meet
5 ASTM Specification D6751-09 for quality. The potential for biodiesel utilization in diesel fuel is much
6 greater in the United States than the mandated targets. However, biodiesel production is feedstock limited,
7 and much research into non-traditional and novel feedstocks is being conducted [8], [9], [10], [11], [12],
8 [13], [14], [15].

9 ASTM D6751-10 was modified in 2012 to include a No. 1-B grade, which placed a voluntary maximum
10 limit on monoglycerides (monoacylglycerols, or MGs) of 0.40 weight percent (wt%) in an effort to improve
11 cold weather operability of biodiesel blends. This limit on the B100 is intended to prevent operability
12 problems in blends of up to 20 volume percent biodiesel (B20) in petroleum diesel fuel. Several studies
13 have focused on MG properties and how these compounds can negatively impact low-temperature
14 operability of biodiesel and biodiesel blends [16], [17], [18], [19].

15 The measurement of glycerides in biodiesel is based on the work of Plank and Lorbeer in 1995 and has
16 been standardized in ASTM method D6584-17 [20], [21]. The method includes identification of specific
17 MGs (monopalmitin, monostearin, monoolein, monolinolein, and monolinolenin) and was based on the
18 assumption that these are the only MGs of interest. In addition, the chromatography does not resolve
19 monoolein, monolinolein, and monolinolenin, which co-elute as a single peak. Diglycerides
20 (diacylglycerols) and triglycerides (triacylglycerols) are not individually quantified, but rather are identified
21 as groups of peaks that elute within specific retention time regions relative to an internal standard.

22 D6584-08 was modified in 2010 to include a non-mandatory appendix, providing information to users to
23 improve peak identification and these revisions were first published as D6584-10. This study examines
24 further improvements to the most current version of D6584, D6584-17, for MG determination without
25 making major changes to the method, such as column choice, temperature ramp, and injection strategy, in

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1 an effort to determine if a significant improvement could be made while preserving as much of the current
2 method as possible. We hypothesized that errors in MG determination occur due to primarily two causes.
3 First, newer technology gas chromatographs (GCs) with improved flow control, modern columns, and the
4 option to operate in constant flow or constant pressure modes can strongly impact the chromatography and
5 results of this method. Second, new feedstocks can contain significant quantities of fatty acids (FAs) other
6 than C16 and C18 and therefore contain MGs other than those listed in the method that need to be quantified
7 for accurate determination of overall MG concentration. For biodiesel produced from these novel and non-
8 traditional feedstocks, the current method does not give an accurate quantification of bonded glycerin or
9 MG content simply because it does not properly account for the MGs, beyond C16 and C18 species, present
10 in the biodiesel. The improvements proposed in this study should also lead to more accurate determination
11 of diacylglycerols and triacylglycerols, ultimately improving the overall accuracy of the method for total
12 glycerin determination, though this is outside the scope of the current work.

13 Biodiesel prepared from field pennycress (*Thlaspi arvense* L.) oil contained 32.8 wt% 13*Z*-docosenoic acid
14 methyl ester (C22:1), along with other constituents such as 11*Z*-eicosenoic acid methyl ester (C20:1, 6.8
15 wt%) [8]. The fatty acid methyl ester (FAME) profile of this biodiesel is significantly different from
16 commercially available biodiesels, and it follows that the MGs will also be significantly different from the
17 five MGs identified by the D6584-17 method. Algal-oil-derived biodiesel is another feedstock in which
18 non-traditional FAME profiles may be encountered. The properties of oils from different algal species vary
19 greatly [13].

20 A 2010 review by Mata et al. [12] listed 44 strains of microalgae and noted that the wide range of FA
21 profiles of the species may have significant impacts on the biodiesels produced. Gouveia and Oliveira [14]
22 highlighted six algal species in their work. The FA profiles of these species showed a measurable
23 contribution of unsaturated C16 constituents, which are not found in current feedstocks and not quantified
24 by D6584-17. One species, *Nannochloropsis* sp., contained a high level of C20:5, which is also not found
25 in traditional feedstocks and thus is not accounted for by ASTM D6584-17.

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1 In an effort to correct the deficiencies of ASTM D6584-17 discussed above, we developed approaches for
2 quantification of MGs with even FA chain lengths ranging from C10 to C22 using a modern column and
3 GC operating in constant-flow mode.

4 2. METHODS

5 A sample of soybean-derived biodiesel was obtained from Minnesota Soybean Processors (Brewster,
6 Minnesota). The producer provided a certificate of analysis for this sample, including MG content. We
7 determined that the MG content of the sample was 0.419 wt%. The biodiesel met all other D6751 properties.
8 Arugula [*Eruca vesicaria* (L.) Cav. subsp. *sativa* (Mill.) Thell.] and cress (*Lepidium sativum* L.; cv.
9 "Cressida") seeds were purchased from Johnny's Selected Seeds (Winslow, Massachusetts). Camelina
10 (*Camelina sativa* L.) seeds were purchased from Marx Foods (Atlantic Highlands, New Jersey). Cuphea
11 seeds from germplasm line PSR 23 (*Cuphea viscosissima* × *C. lanceolata*) were obtained from U.S.
12 Department of Agriculture test plots in Peoria County, Illinois, and oil was mechanically expelled as
13 described previously [22]. Field pennycress (*T. arvense* L.) seeds were collected from U.S. Department of
14 Agriculture test plots in Peoria County, Illinois, and oil was mechanically expelled as described previously
15 [10]. Cold-pressed meadowfoam (*Limnanthes alba* L.) seed oil was purchased from Natural Plant Products,
16 Inc. (Salem, Oregon). Wild mustard (*Brassica juncea* L.) seeds were harvested from mature plants growing
17 on fallow land on the campus of the Universidade Federal de Viçosa (Viçosa, Brazil). Dried arugula,
18 camelina, cress, and wild mustard seeds were ground in a coffee grinder, and oil was extracted by Soxhlet
19 with high-performance liquid chromatograph grade hexanes (mixture of isomers) for 24 h. Hexanes were
20 removed by rotary evaporation at 10 mbar and 30°C to yield crude arugula, camelina, cress, and wild
21 mustard oils. All reagents were obtained from Sigma-Aldrich Corp (St. Louis, Missouri) and used as
22 received.

23 2.1 Mineral Acid-Catalyzed Pretreatment

24 The acid value of the oils was measured in triplicate with means reported following American Oil Chemists'
25 Society official method Cd 3d-63 (reapproved 2009) [23]. Plant oils with acid values above 0.50 milligram

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1 of potassium hydroxide per gram of oil (mgKOH/g) were pretreated to render them more amenable to
2 homogeneous alkaline-catalyzed methanolysis and included crude arugula (acid value 0.54 mgKOH/g),
3 camelina (2.06 mgKOH/g), cress (0.89 mgKOH/g), and field pennycress (0.61 mgKOH/g) oils. Acid values
4 of the remaining oils were as follows: soybean – 0.03 mgKOH/g; wild mustard – 0.44 mgKOH/g; cuphea
5 – 0.45 mgKOH/g; and meadowfoam – 0.07 mgKOH/g. Mineral acid-catalyzed conversion of the free FAs
6 to methyl esters was performed as a pretreatment. This was accomplished in a 1-L three-necked round-
7 bottom flask connected to a reflux condenser and a magnetic stirrer set to 1,200 rotations per minute.
8 Initially, the plant oil and methanol (35 volume percent) were added to the flask, followed by drop-wise
9 addition of sulfuric acid (1.0 volume percent). The contents were heated with stirring at reflux for 4 h. Upon
10 cooling to room temperature, the phases were separated. The upper oil phase was washed with distilled
11 water until a neutral pH was achieved, followed by rotary evaporation at reduced pressure (20 mbar; 30°C)
12 to remove residual methanol. Finally, drying with magnesium sulfate ($MgSO_4$) yielded the acid-pretreated
13 plant oils. The final acid values of arugula, camelina, cress, and field pennycress oils after pretreatment
14 were 0.09, 0.36, 0.11, and 0.09 mgKOH/g, respectively.

15 **2.2 Methanolysis**

16 Methanolysis of acid-pretreated arugula, camelina, cress, and field pennycress oils as well as crude cuphea,
17 meadowfoam, and wild mustard seed oils was conducted in a 1-L three-necked round-bottom flask
18 connected to a reflux condenser and a magnetic stirrer set at 1,200 rotations per minute. Initially, the plant
19 oil and methanol (6:1 mole ratio) were added and heated to 60°C (internal temperature monitored by digital
20 temperature probe), followed by the addition of sodium methoxide catalyst (0.50 wt% with respect to oil).
21 After reacting for 1.0 h, the mixture was equilibrated to room temperature and the lower glycerolic phase
22 was removed by gravity separation (>2 h settling time), followed by removal of methanol from the ester
23 phase by rotary evaporation at reduced pressure (10 mbar; 30°C). The crude products were washed with
24 distilled water until a neutral pH was obtained and dried with $MgSO_4$ to yield FAME. Yields of the esters
25 are as follows: arugula oil methyl esters (ARME) 98 wt%, camelina oil methyl esters (CAME) 94 wt%,

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cress oil methyl esters (CRME) 98 wt%, cuphea oil methyl esters (CUME) 92 wt%, field pennycress oil methyl esters (FPME) 97 wt%, meadowfoam oil methyl esters (MFME) 97 wt%, and wild mustard oil methyl esters (WMME) 89 wt%.

2.3 Fatty Acid Profile

The FAME profiles of the B100s were measured using an Agilent (Palo Alto, California) model 7890A GC with flame ionization detection (FID) following the EN14103 method using a 60-m polyethylene glycol column (60 m × 0.25 mm, 0.25 µm df) [24]. A mixture of FAME (GLC-90) standards purchased from Nu-Chek Prep (Elysian, Minnesota) was analyzed to determine the retention times of individual components.

2.4 Monoglyceride Determination

All biodiesel samples were initially derivatized with N-methyl N-trimethylsilyltrifluoracetamide following the procedure outlined in section 10.1 of D6584-17. The MG analysis was conducted using an Agilent model 7890A GC-FID. A 5% phenylpolydimethyl-siloxane column (14 m × 0.53 mm, 0.16 µm df) was used along with an integrated 2-m guard column (Restek Part #70289). The GC operating conditions were taken from the D6584-17 method. The analysis used a 1-µL sample injection into a cool on-column injector. The GC was operated in constant flow mode, with helium carrier gas set at 3 mL/min. The FID temperature was 380°C. The GC oven ramp was: initial temperature of 50°C, with a 1-min hold; the first ramp was 15°C/min to 180°C; then the oven temperature was increased at 7°C/min to 230°C; and the final ramp was 30°C/min to 380°C, with a 10-min hold at the final temperature.

A mixture of MGs was prepared using pure compounds purchased from Nu-Chek Prep. An MG stock standard was prepared in pyridine at approximately 2 to 5 mg/mL of each component, and calibration standards were subdivided and derivatized following the procedure described in section 9 of ASTM D6584-17. Calculations of the concentrations of these calibration compounds and determination of their correlation coefficients and calibration functions followed the procedure outlined in section 9 of D6584-17. The mixture included all even saturated chain length MGs (SMGs) from C10:0 to C22:0 and selected monounsaturated MGs (Table 1).

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1 The response of the monounsaturated MG was used to determine the concentration of all unsaturated MGs
2 (USMGs) at a given chain length. It was assumed that the inability of the chromatography to resolve the
3 C18:1, C18:2, and C18:3 USMGs would also result in the inability to separate USMGs of other chain
4 lengths, so only the monounsaturated MGs were used in the calibration. Fig. 1 shows a chromatogram of
5 these MGs, along with the internal standard from the D6584-17 method under our operating conditions.

6 Table 1. Monoglycerides Used in Standard Mixture

Monoglyceride	Shorthand
Monocaprin	C10:0
Monododecanoin (monolaurin)	C12:0
Monomyristin	C14:0
Monopalmitolein	C16:1
Monopalmitin	C16:0
Monoolein	C18:1
Monostearin	C18:0
Monoeicosenoin	C20:1
Monoarachidin	C20:0
Monoerucin	C22:1
Monobehenin	C22:0

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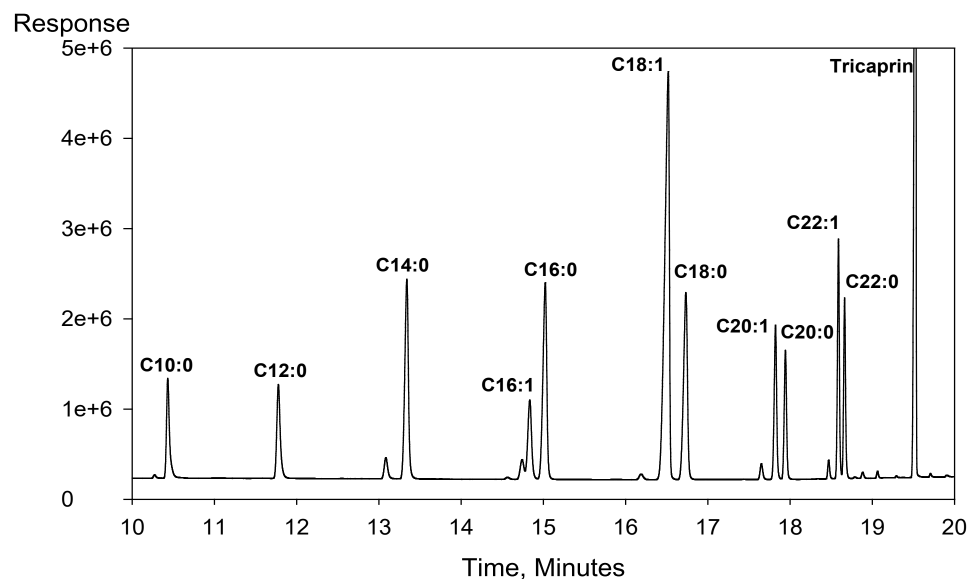

Fig. 1. Representative chromatogram of a standard C10–C22 MG mixture consisting of even carbon chain (saturated and selected monounsaturated) MGs

2.5 Constant Pressure Compared to Constant Flow Operation

The D6584 method, which was developed in the mid-1990s (originally published as ASTM D6584-00), does not specify constant pressure or constant flow operation. The use of advanced electronic pressure control was not common in GCs, and many early users ran the method with constant pressure operation. This influenced the relative retention time (RRT) windows published in D6584-00. Most new GCs allow selection of constant flow or constant pressure operation. The main drawback to constant pressure operation for this method is the change in carrier gas viscosity over the large temperature range. As the oven temperature increases, the viscosity of the carrier gas increases, resulting in decreased flow if the column head pressure is not increased to compensate. This results in a significant decrease in the linear velocity of the carrier gas, leading to later retention times, peak broadening, and loss of resolution. For this reason, all work in this paper was performed under constant flow operation.

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1 **3. RESULTS AND DISCUSSION**

2 **3.1 MG Identification**

3 MGs were identified by retention time matching to the standard mixture, contrary to the procedure in
4 D6584-17, which uses RRT windows compared to added internal standards to identify the compounds of
5 interest. In D6584-17, the RRT windows for the glyceride peaks are defined relative to the second internal
6 standard, tricaprin. For example, monopalmitin is given an RRT of 0.76. Thus, the ratio of the retention
7 time of tricaprin to the retention time of monopalmitin should be 0.76. However, under our conditions the
8 ratio is 0.78. This difference in RRT may seem slight, but the result is a difference in expected retention
9 time of 0.4 minute compared to the retention time that would be used to identify this compound. The
10 drawback with using fixed RRTs to determine peaks of interest is that differences in column manufacturing
11 and GC instrumentation can alter this ratio, even when following the temperature program in the method
12 exactly. As an example, Fig. 2 shows a chromatogram generated from our instrument for standards of C16:0,
13 C18:1, and C18:0 MGs compared to the RRT windows provided in D6584-17 (note D6584-17 only
14 calibrates for C18:1, not C16:0 and C18:0. Detail on identifying these peaks is provided in the D6584-17
15 appendix). These differences are attributed to several factors, which include improved columns since the
16 method was first published, improved GCs, and constant flow operation. Using the RRT windows in the
17 method would result in underreporting of the MGs even for conventional C16/C18 biodiesel, as the
18 monopalmitin and monostearin peaks fell outside of these method-prescribed windows on our instrument.

19 The concentrations of the MGs were determined from individual component calibration functions using the
20 calculations presented in Section 11.1.2 of ASTM D6584-17. Our limit of detection was estimated at 0.010
21 wt% MGs, with a limit of quantitation (LOQ) of 0.020 ± 0.010 wt% MGs. The LOQ was determined by
22 multiple preparations and injections of three different B100s with various levels of MGs. Each B100 was
23 prepared five times and injected to determine our variability on replicate analysis of the same sample.

24

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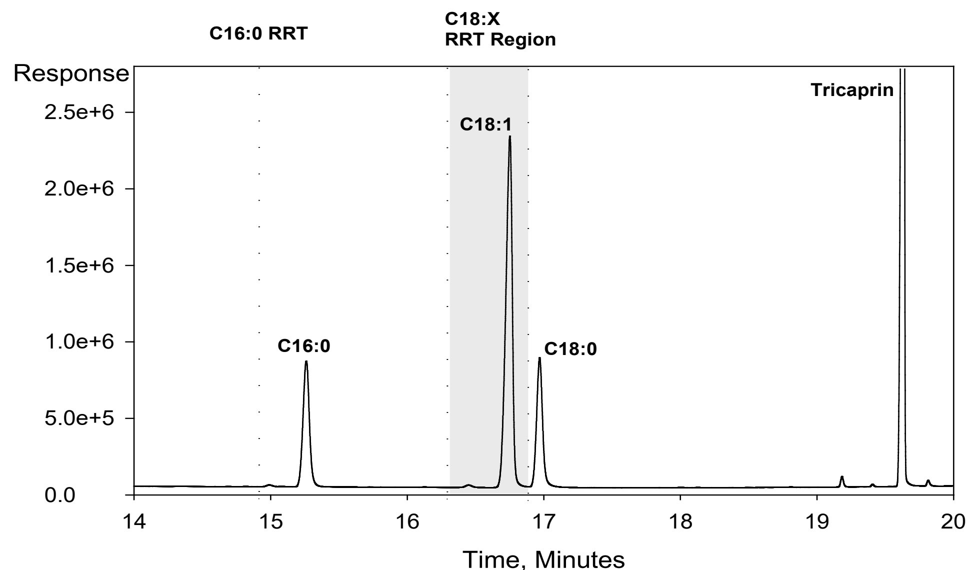

Fig. 2. Standards of monopalmitin, monoolein, and monostearin compared to RRT windows provided in the D6584-17 method

The B100s from all of the feedstocks were analyzed for their FAME profiles to determine the major components (second step of the improved process). Table 2 lists the measured FAME profiles of the B100s in this study, showing significant contribution to the total FAME distribution by non-C16 and C18 fatty esters for the biodiesels produced from the non-traditional feedstocks.

Table 2. FAME Distribution Analysis Results for Biodiesel Samples

FAME, %	SME (Soy)	ARME (Arugula)	WMME (Wild mustard)	CAME (Camelina)	CRME (Cress)	CUME (Cuphea)	FPME (Field pennycress)	MFME (Meadowfoam)
C10:0	<0.1	<0.1	<0.1	<0.1	<0.1	57.36	<0.1	<0.1
C12:0	<0.1	<0.1	<0.1	<0.1	<0.1	2.33	<0.1	<0.1
C14:0	<0.1	<0.1	<0.1	<0.1	<0.1	3.45	<0.1	<0.1
C16:0	11.12	3.92	2.41	5.41	7.94	5.23	2.29	0.17
C16:1	<0.1	0.22	0.18	<0.1	0.24	<0.1	0.18	<0.1

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FAME, %	SME (Soy)	ARME (Arugula)	WMME (Wild mustard)	CAME (Camelina)	CRME (Cress)	CUME (Cuphea)	FPME (Field pennycress)	MFME (Meadowfoam)
C18:0	4.77	1.09	0.84	2.61	2.60	0.82	0.41	<0.1
C18:1	21.88	15.66	7.24	18.24	27.16	9.80	11.43	0.39
C18:2	53.68	7.8	13.22	17.51	7.06	5.54	18.28	<0.1
C18:3	8.85	11.23	11.77	27.75	28.53	0.48	5.54	<0.1
C20:0	<0.1	0.74	0.76	1.64	2.41	<0.1	<0.1	0.84
C20:1	<0.1	9.57	5.17	13.04	11.09	<0.1	9.57	61.58
C20:2	<0.1	<0.1	0.91	1.28	0.35	<0.1	1.60	<0.1
C22:0	<0.1	0.96	0.85	<0.1	0.62	<0.1	<0.1	<0.1
C22:1	<0.1	<0.1	44.01	<0.1	3.39	<0.1	33.82	12.63
C22:2	<0.1	41.38	1.77	0.84	<0.1	<0.1	0.58	15.11
C24:1	<0.1	1.55	2.31	<0.1	<0.1	<0.1	3.42	<0.1
Unidentified	0.0	1.57	3.52	2.99	1.88	<0.1	5.32	5.93
Total FAME	100.3	95.7	95.0	91.3	93.3	85.0	93.3	96.7
ΣSaturated FAME	15.9	6.72	4.86	9.66	13.58	69.19	2.70	1.01
% Saturated FAME of total FAME	15.8	7.02	5.11	10.58	14.56	81.40	2.89	1.05

Our most recent B100 quality survey [4] determined the percent saturated FAME in the total FAME (percent saturated FAME) and the percent SMG in the total MG (% SMG) in over 50 commercial B100 samples.

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This data showed that percent SMG was nearly equal to percent saturated FAME. The current study assumes this relationship to be true, within the precision of both methods.

3.2 MG Analysis

Each biodiesel was analyzed for FAME profile and MG content. The MG content was analyzed using the published D6584-17 method and reanalyzed using our modifications to the method. To simplify the discussion, the following terms are defined.

MG_P: MG content, wt%, using D6584-17 method, as published

SMG_P: SMG content, wt%, using D6584-17, without modification

% SMG_P: Ratio of SMG_P to MG_P, converted to percent

MG_M: MG content, wt%, using our modifications to D6584-17

SMG_M: SMG content, wt%, using our modifications to D6584-17

%SMG_M: Ratio of SMG_M to MG_M, converted to percent

% Saturated FAME: Ratio of saturated FAME to total FAME, converted to percent

Table 3 summarizes the results from all samples in this study using the published D6584-17 methodology for MG quantification based on the calibration function of C18:1 and using the RRT windows for MG identification. These results are compared to results generated using our methodology of utilizing the FAME profile of the B100 to guide identification of MGs along with the actual retention times of individual MG standards. Individually identified MGs are quantified based on our calibration. The percent saturated FAME (of total measured FAME) is also provided.

Table 3. Monoglyceride Quantitation of Traditional and Non-Traditional Biodiesels, by Published D6584-17 Methodology and by Modified D6584-17 and FAME Methodology.

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Biodiesel	MG_P by published method	% SMG_P of total MG by published method	MG_M by modified method	% SMG_M of total MG by modified method	% Saturated FAME
SME	0.362	6.6%	0.414	18.2%	15.8%
ARME	0.044	38%	0.028	<1%	7.0%
WMME	0.108	19%	0.235	8.6%	5.1%
CAME	0.245	9.7%	0.345	10.2%	10.6%
CRME	0.234	13.1%	0.322	13.9%	14.6%
CUME	0.174	5.3%	0.820	79.9%	81.4%
FPME	0.020	100%	0.066	100%	2.9%
MFME	0.038	39%	0.004	<1%	1.1%

1 MG_P and SMG_P are calculated from the published method, with no deviations. MG_M and SMG_M are calculated from the modified
2 D6584-17 methodology, including the use of FAME profile to identify major components and a standard MG mixture to identify
3 retention times of MGs and SMGs.

4 **3.3 Biodiesel Produced from Conventional Feedstocks**

5 In our analysis, soybean-derived biodiesel (SME) was used as a surrogate for any biodiesel produced from
6 conventional feedstocks, such as animal fats, canola, corn oil, etc. The ratios of C16 and C18 peaks will
7 change based on the feedstock, but the analysis is similar [22].

8 Using the RRT windows in D6584-17 for MG identification, the MG_P content of the SME biodiesel was
9 0.362 wt%, with 6.6% SMG_P, while the percent saturated FAME was 15.8%. This difference in SMGp and
10 percent saturated FAME indicated that the concentration of SMG determined by the published method was
11 lower than the actual amount. For SME, the major MG components were C16:0, C18:0, and the co-eluting
12 peak of C18:1, C18:2, and C18:3 (Fig. 3). Using actual retention times to identify these MGs rather than
13 the RRT windows resulted in an MG_M concentration of 0.414 wt%. The SMG_M content was 18% of total
14 MG_M using this technique, which was much closer to the 16% saturated FAME in the biodiesel.

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1 One of the difficulties in accurately identifying the correct peaks in the glyceride analysis was the large
2 number of unknown peaks compared to the peaks of interest. As Fig. 3 shows, there were multiple peaks
3 present that were not MGs and were not considered in the determination. The large peak eluting between
4 the C10:0 and C12:0 MGs was FAME, while the identity of the other peaks is unknown. Any compound
5 found in biodiesel with a labile hydrogen will be derivatized by N-methyl N-trimethylsilyltrifluoracetamide.
6 Many of these peaks are also separated by this column and picked up by the detector. The difficulty is in
7 determining which peaks are MGs and which peaks are other trace compounds in biodiesel, such as sterols,
8 free FAs, wax esters, etc.
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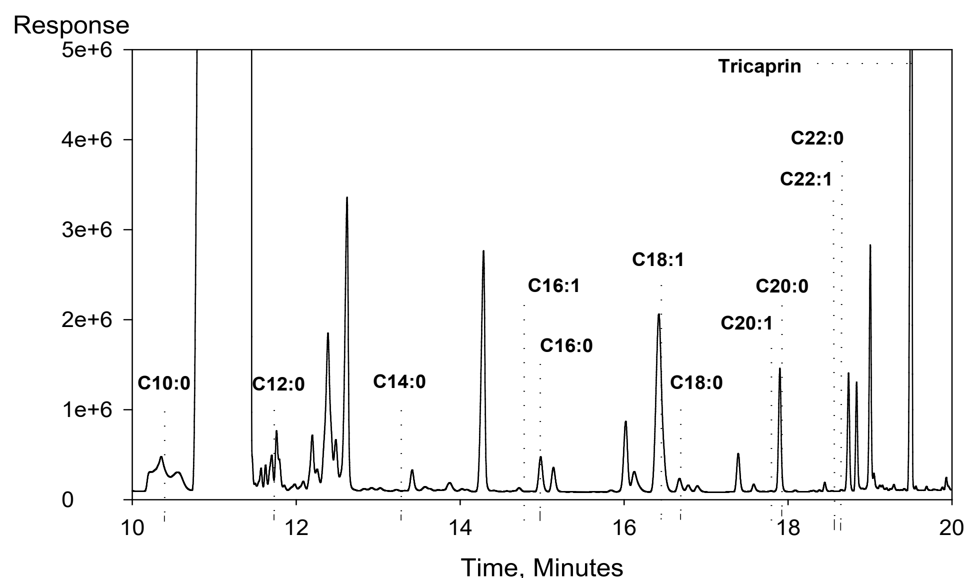

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11 **Fig. 3.** Chromatograms of SME with all MG retention times labeled. Only peaks from C16:0, C18:1, and C18:0 were
12 used in the analysis. The peaks at C10:0, C12:0, and C20:0 were omitted from the calculation due to their absence in
13 the FAME analysis.

14 **3.4 Biodiesel from Non-Traditional Feedstocks with MG Content Above LOQ**

15 **Cuphea Oil Methyl Esters:** The MG_P concentration of CUME was 0.174 wt%, with 5.3% SMG_P following
16 the published methodology. From the measured FAME profile of CUME, which indicated that the sample
17 was 81% saturated FAME, it was hypothesized that the SMGs should be around 80% of the total MGs. The

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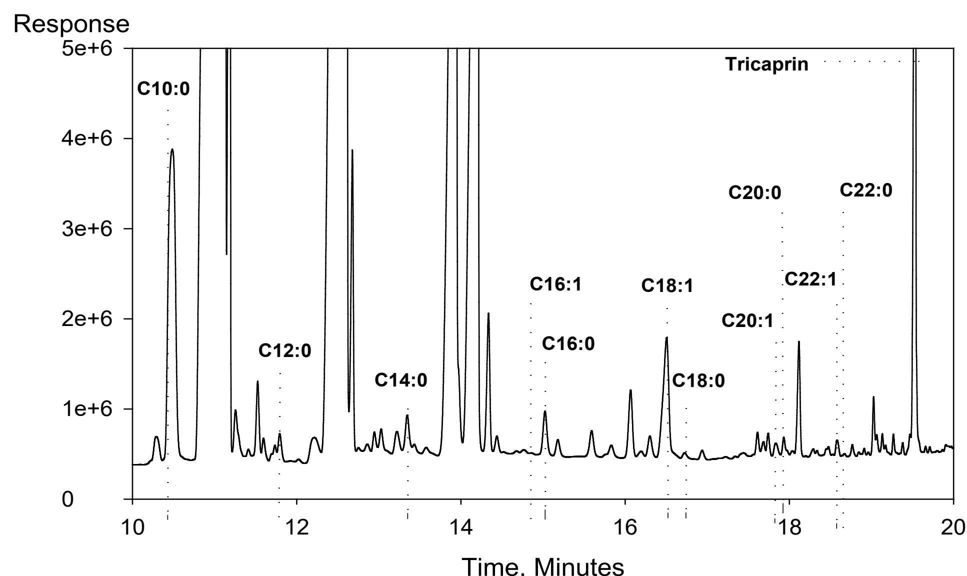
1 high C10:0 content of cuphea B100 (57% of total FAME) should result in a significant amount of C10:0
2 MGs present in the biodiesel (Fig. 4). By referencing the MG standard mixture and only including relevant
3 MGs for CUME (C10:0, C12:0, C14:0, C16:0, C18:1, and C18:2), the MG_M content increased to 0.820
4 wt%. Our improved method determination resulted in SMG_M that was 80% of the total MG_M, compared to
5 5.3% with the published method.



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7 **Fig. 4.** Chromatogram of CUME with MG peaks identified

8 **3.5 Biodiesel from Non-Traditional Feedstocks with MG Content near LOQ**

9 **Arugula Oil Methyl Esters:** Fig. 5 shows a chromatogram of the arugula-derived biodiesel with the MG
10 retention times noted. The MG_P concentration was 0.044 wt%, with less than 0.020 wt% SMG_P
11 (approximately 38% SMG_P). The FAME profile indicated that the biodiesel contained approximately 7%
12 saturated FAME (C16:0, C18:0, with less than 1% from C20:0 and C22:0). The MG_M content dropped to
13 0.028 wt% when using our proposed modifications to the method, including the FAME profile to identify
14 major components in the biodiesel, and the SMG_M became less than 1% of the total MGs. Clearly, even
15 with a detailed FAME analysis, the accurate measurement of MGs can be challenging, as shown in this case
16 due to MG concentrations near the method's LOQ.

17

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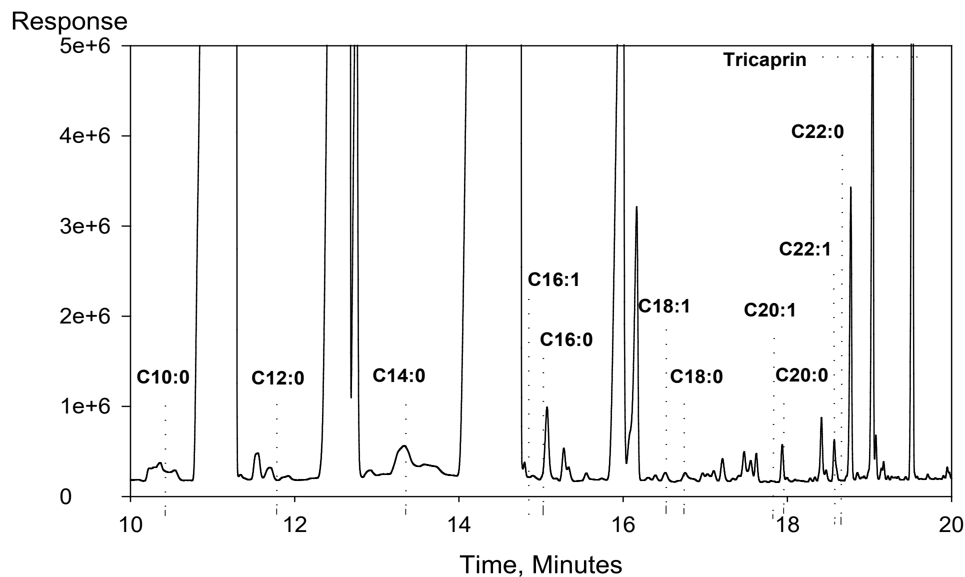

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4 **Fig. 5.** Chromatograms of ARME with MG retention times labeled.

5 Using the same methodology, the remaining B100 samples (WMME, CAME, CRME, FPME, and MFME)
6 were analyzed for MG content. These chromatograms are provided in the supplemental material (see Figs.
7 S1–S5).

8 The analysis of the wild mustard B100 (WMME) was similar to the analysis of the cuphea B100 (CUME),
9 where MG content was above the method LOQ. For the WMME, 56% of the fatty acid profile was
10 composed of C20 and C22 FAME, ensuring that any significant contribution from the relevant MGs would
11 be ignored using the method as published. Using the modified methodology, the total MG_M content of the
12 sample increased by 50%, and the SMG_M content decreased. The SMG_M of the WMME (8.6%) agreed well
13 with the saturated FAME content of the sample (5.1%).

14 Analysis of the CAME and CRME samples resulted in no net change in the SMG content using the
15 published and modified methods. The camelina and cress B100s contained FAME outside of the C16 and
16 C18 range but in lower concentrations than the other B100s discussed. The CAME sample was composed
17 of 21% C20 FAME, with no C22 FAME detected. The CRME sample was composed of 19% C20 and C22

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1 FAME. In these samples, the published method captured almost 80% of the MGs. However, for the most
2 accurate determination of MG concentration, consideration of the FAME profile and a standard MG curve
3 for C20 and C22 MGs is recommended.

4 The FPME may contain wax esters, which typically do not transesterify as efficiently [25]. This sample
5 also contained large peaks in the retention time region where phytosterols are typically detected. Although
6 not the subject of this work, phytosterols were also qualitatively detected through modification of D6584-
7 17. The chromatographic conditions were the same as discussed above, and a standard of phytosterols,
8 prepared in the same manner as the MGs, was used to determine elution time (Fig. S4). Phytosterols were
9 not quantified in this work. In addition, other trace components present in the sample may overlap with the
10 peaks of interest, leading to overestimation of the MGs in the sample. This is most likely the case for the
11 FPME, which our analysis showed as 100% SMG_M, when in fact the sample was less than 10% saturated
12 FAME.

13 For MFME, nearly 90% of the FAME comes from three species: C20:1, C22:1, and C22:2. The MG_P
14 concentration was 0.038 wt%. By using our method modifications, we found the MG content dropped by
15 an order of magnitude (MG_M = 0.004 wt%). Our analysis determined that the SMG_M was similar to the
16 percent saturated FAME. We recommend performing this analysis on a wide variety of novel feedstocks
17 with significant MG content to ensure the accuracy and robustness of our proposed improvements.

18 **4. CONCLUSIONS**

19 The ASTM D6584-17 method was used to determine the MG content of seven biodiesels derived from
20 novel feedstocks (WMME, CAME, CRME, CUME, FPME, MFME) and a commercial soybean-derived
21 biodiesel [SME]. The SME sample contained only C16 and C18 FAME, while the other samples derived
22 from non-traditional feedstocks contained a wider range of FAME from C10:0 to C24:1. Each sample was
23 analyzed by D6584-17, as published, and using our proposed modifications to better identify MGs. One
24 significant point is that the method was run in constant flow mode. The goal of our modifications was to
25 improve the accuracy of D6584-17 without making changes to the chromatography. Our modifications

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included running a FAME profile for each biodiesel and making the assumption that the percent saturated FAME in the biodiesel would be roughly equal to the saturated MG content (as a percent of total measured MG content) of the biodiesel. When these values were roughly equal, we determined that D6584-17 accurately quantified MG concentration.

Of the seven biodiesel samples, three had roughly the same concentration of SMG when analyzed with and without the proposed modifications (CAME, CRME, and FPME). For two of these samples, determining the FAME profile allowed us to more accurately quantify MG concentration (CAME and CRME). For the third sample (FPME), the MG concentration was near the LOQ for the method. The modifications improved the MG quantification of the four other samples, as evidenced by near equality of the percent SMG and percent saturated FAME in the samples.

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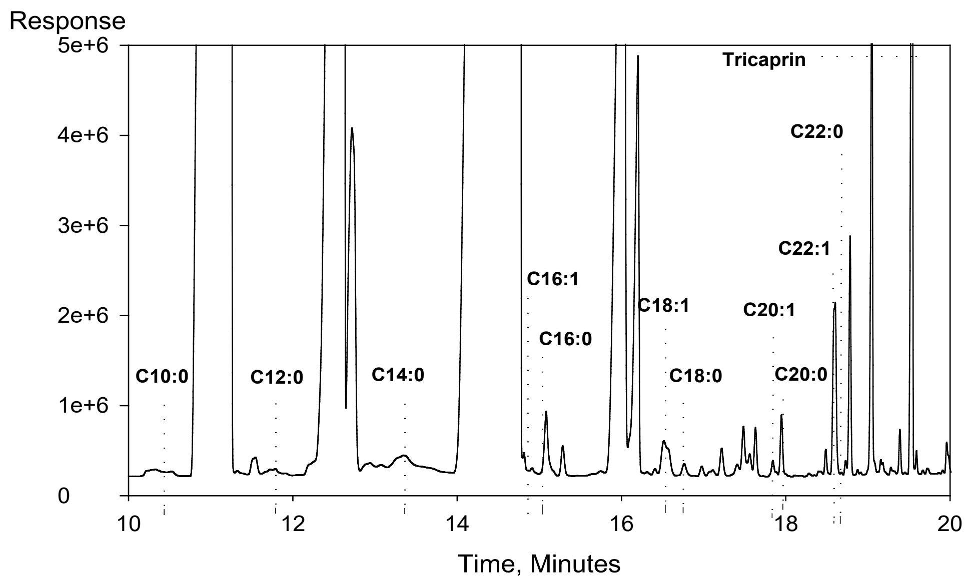

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5 Fig. S1. Chromatogram of WMME with MG retention times labeled.

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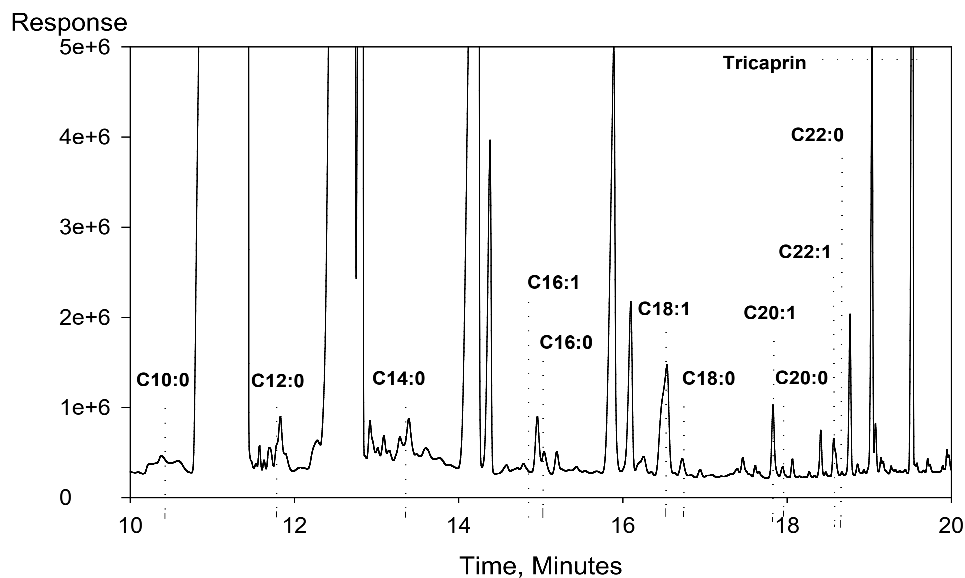

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8 Fig. S2. Chromatogram of CAME with MG retention times labeled.

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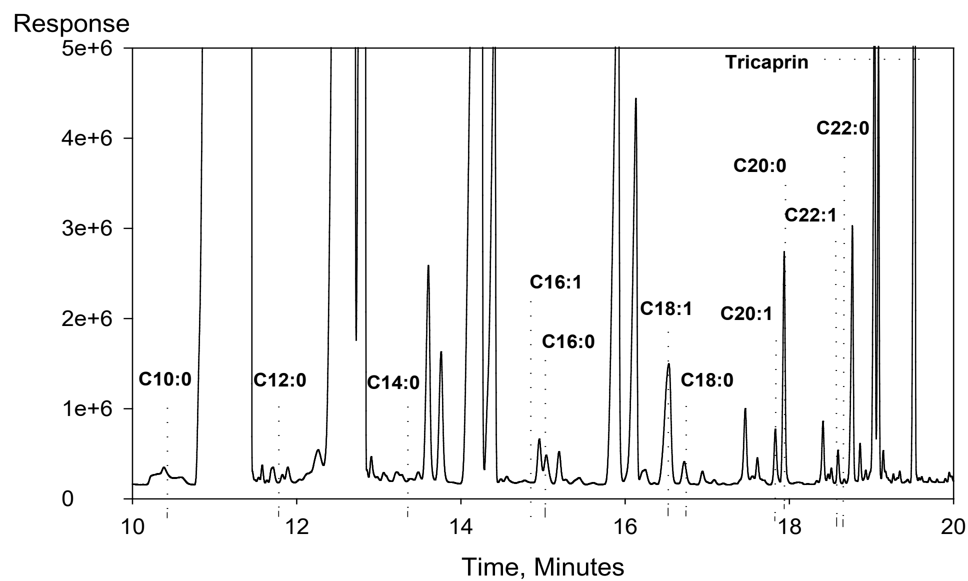

2 Fig. S3. Chromatogram of CRME with MG retention times labeled.

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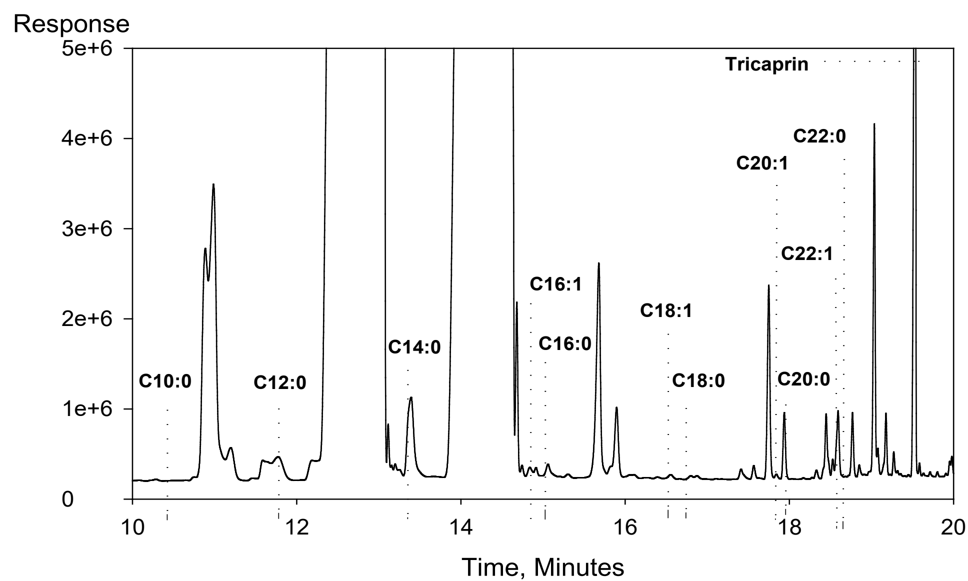

5 Fig. S4. Chromatogram of FPME with MG retention times labeled.

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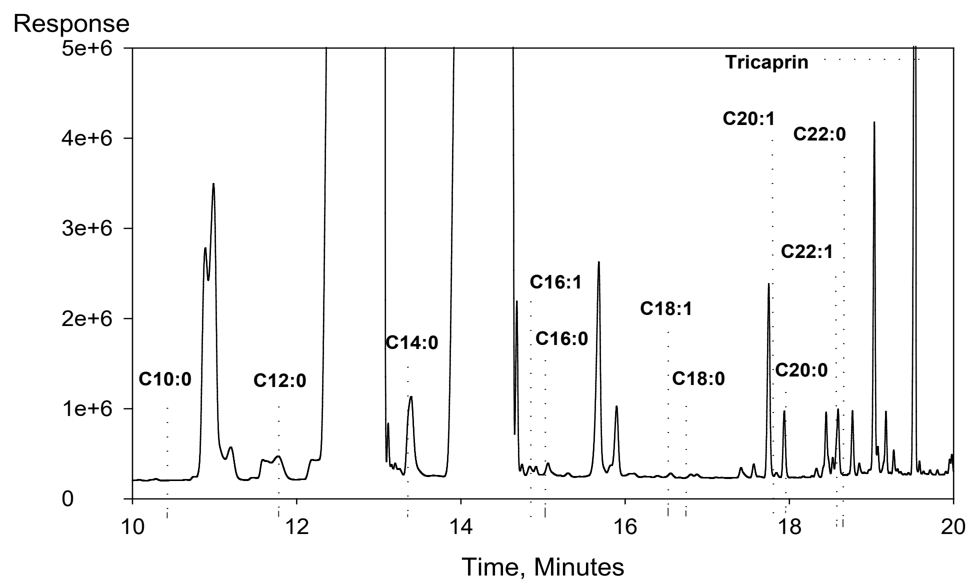

2 Fig. S5. Chromatogram of MFME with MG retention times labeled.

3

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