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Simultaneous High-Temperature GC-FID/MS Analysis of Monocarboxylic Acids and Acylglycerols in Biofuels and Biofuel Intermediate Products

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Abstract

Triacyl-, diacyl- and monoacylglycerols (TAGs, DAGs, MAGs) along with monocarboxylic acids (MCAs) are intermediate products in many triacylglycerol oil-to-biofuel conversion pathways. Accumulation of these compounds leads to poor biofuel characteristics and may result in fuel system damage. We developed a method for simultaneous identification and quantification of a wide range of MCAs (C₄-C₁₈), MAGs, DAGs, and TAGs. The method is based on trimethylsilylation followed by high temperature GC with programmed temperature vaporizer (PTV) injection coupled to parallel FID and MS detectors (HTGC-FID/MS).

To minimize the discrimination of both low and high molecular weight species typically occurring on the injector, we optimized injection conditions using a central composite design. The critical variables were the time at initial temperature (40 °C), splitless time, and the interaction between these two parameters. Among three tested electron ionization source/quadrupole analyzer temperatures, a 350/200 °C setting provided the highest response and signal-to-noise ratio for TAGs and did not have an effect on MAGs and DAGs.

Similar results were obtained when quantifying target analytes in intermediate products of soybean oil cracking with FID and MS (using specific acylglycerol fragmentation ions). The instrumental FID limits of detection (LODs) were 0.07–0.27 ng for most of the target analytes. Selected ion monitoring (SIM) LODs were 0.01–0.05 ng for MCAs and 0.03–0.14 ng for acylglycerols. For the total ion current (TIC), LODs observed increased with acyl chain length and degree of unsaturation, resulting in an increase from 0.05 to 0.18 ng for MCAs (C₅ to C₁₈) and from 0.03 to 1.8 ng for acylglycerols (TAGs C₈ to C₂₂). Deviations in the repeatability of sample preparation, intra- and inter-day analyses, including sample stability over an eight-day time period, did not exceed 10% variance. From these results we conclude that the developed method is accurate and robust for the determination of the target compounds from organic mixtures such as acylglycerols and MCAs produced during the processing of TAGs into biofuels.

Keywords

Acylglycerols, monocarboxylic acids, biofuels, gas chromatography, mass spectrometry, DOE

1. Introduction

Biofuels produced from natural sources of triacylglycerols (TAGs), such as vegetable oils, algae, or bacteria have gained popularity as a feedstock for the production of renewable transportation fuels [1]. Common ways to convert TAGs feedstock into biofuels are: transesterification with an alcohol, hydroprocessing, and thermal cracking (i.e., pyrolysis with or without a catalyst) [1, 2]. The organic liquid product (OLP) generated in the primary reaction step of many of these processes may still contain un-reacted starting material (i.e., TAGs) or acidic intermediates such as mono- and diacylglycerols (MAGs and DAGs), free glycerol [1, 2], high molecular weight (MW) monocarboxylic acids (MCA) (particularly C₁₆–C₁₈, originally present in the feedstocks as triesters of glycerol), and free (C₄–C₁₈) MCAs [3].

The occurrence of these undesired species in the primary biofuel product can create problems during biofuel storage, cause damage to engines and fuel tanks from corrosion, and affect fuel system performance by filter clogging and injector fouling [4]. Moreover, it can negatively affect the yield and degree of subsequent processing required to generate acceptable fuels conforming to standard specifications such as those listed in ASTM D 6751 or EN-14214. Therefore, speciation characterization is essential to optimize biofuel conversion pathways and to ensure that adequate fuel quality is achieved

To comply with applicable fuel standards, multiple analytical methods have been previously developed to selectively determine specific classes of carbonyl compounds of various size ranges, as discussed below. However, none of these methods provide a detailed analysis of both organic acids and acylglycerols in one run, partially due to the wide molecular weight range (from 100 amu for MCAs to 1000 amu for TAGs) of target compounds.

ASTM D664 is a standard method for the determination of a total acid number using potentiometric titration. ASTM D664 was originally developed to measure the content of acidic compounds in petroleum fuel products, most notably reduced sulfur compounds [12]. When applied to TAG-derived fuels, which lack these sulfur compounds, it provides a measure of the total concentration of free fatty acids. However, this method lacks selectivity, as it measures both organic and inorganic acidic components, which may or may not originate from the triacylglycerols during oil-to-biofuel conversion. Moreover, it provides no information on the distribution of the individual MCAs.

By contrast, a GC-FID analysis of derivatized MCAs can provide a detailed speciation of MCAs C₆–C₂₄ [13] and MCA C₁₄–C₁₈. For example, Lau and coworkers demonstrated comparable results and repeatability to titration using GC-FID to characterize MCAs in palm oil [14].

For the analysis of free and total glycerols, i.e., totals of glycerol, MAGs, DAGs and TAGs, derivatization followed by high-temperature GC-FID (HTGC-FID) is often employed using a non-polar stationary phase and cold on-column injection [15-19] following standard methods ASTM D6584 [4] or EN 14105 [20], which utilize *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA) derivatization. Anion-exchange chromatography with pulsed amperometric detection has been proposed to eliminate the need for the derivatization step [21]. However, neither of these methods provides characterization of individual species, which is essential for mechanistic insights during the biofuel development.

When performing a GC analysis of TAGs, the injection technique is one of the critical parameters to ensure a complete transfer of such high molecular weight components onto the column. On-column injection is most often employed [15-19, 22, 23] but may result in column contamination with undesirable non-volatile species from TAG feedstock conversion[24]. A programmed temperature vaporizer (PTV) can be used to achieve transfer efficiencies comparable to on-column injection for high molecular weight species while minimizing the introduction of non-volatile species. Several research groups have reported the application of PTV for the analysis of highly concentrated

samples of synthetic and food fat TAG mixtures [24-31]. It should be noted that a slight discrimination of larger TAGs was observed during the use of splitless PTV injection [24-30]. This was assumed to be due to insufficient elution out of the syringe needle or irreversible adsorption to the chromatographic column [24, 32]. Šánek et al. [33] showed the importance of careful optimization of the injection conditions when analyzing fatty acid methyl esters and acylglycerols and developed a method for their simultaneous determination.

A number of chromatographic methods employing a MS detector have been developed over the last couple of decades [23, 34-42] to enable detailed analysis of individual acylglycerol species. Both GC-MS [34-37] and LC-MS [38-42] have been employed for the analyses of compositionally different TAG samples. Yet HTGC has been suggested as a favored technique in a review summarizing the developments in the field of TAG GC and LC analysis over the past decade [23]. The majority of these LC and GC methods have focused on the separation and identification of species in lipid mixtures (i.e., food products) that have high concentrations of TAGs, which allowed to first fractionate samples using thin layer prior to GC or LC analysis [34-36, 38, 41, 42]. A few studies also used a GC equipped with two detectors, i.e., MS and FID, to ensure reliable identification and quantification of TGAs and MCAs [43-47]. To our knowledge, the sensitivity of these methods was not reported, possibly due to the high concentrations of the target analytes.

A few studies reported methods for simultaneous detailed analysis of MCAs and acylglycerols in vegetable oils, animal fats, or biodiesel matrices using GC [13, 14, 48], LC [49-51] or SFC [52, 53] (Suppl. Table 1), all with various limitations. The HTGC-FID methods did not allow for an accurate identification of heterogeneous DAGs and TAGs [13, 14], whereas the reported GC-MS applications [31, 48] did not use high temperature programs (i.e., HTGC) and thus were unable to address DAGs and TAGs. In the reported LC [49-51] and SFC [52, 53] methods, low MW acids, i.e., below C₁₆ or C₁₈, were not considered. In addition, quantification was not reported in either LC [49-51] or SFC [52, 53] studies.

The aim of the presented work was to develop a method for simultaneous analysis of a wide range of free MCAs, MAGs, DAGs, and TAGs using HTGC coupled with simultaneous FID and MS detection (HTGC-FID/MS), enabling identification of intermediate products in various matrices. A central composite design (CCD) was employed to optimize the PTV injection conditions to minimize the discrimination of low and high molecular weight species. Also, the GC oven temperature program and MS parameters were evaluated. The applicability and efficiency of the developed method was validated using intermediate biofuel samples generated by the partial noncatalytic thermal cracking of soybean oil. Additional parameters evaluated include method inter- and intra-day repeatability and instrumental limits of detection (LOD). Accuracy and precision of quantification was ensured by the use of an appropriate set of internal and recovery standards.

2. Materials and methods

2.1. Materials

All relevant information on the individual compounds used in this study, including manufacturer, molecular formula, weight, and quantification and confirmation ions (m/z), are listed in Table 1. Two TAG standard mixtures were used: 1) GLC-768 (TAGs C₇–C₂₂, Nu-Chek Prep., Inc., Elysian, MN, USA) for the analytical method development, including the determination of LODs, and 2) GLC-437 (TAGs C₁₆, C₁₈, C_{18:1}, C_{18:2}, C_{18:3}, C₂₀, Nu-Chek Prep., Inc.) for method evaluation and quantification of OLP samples. To account for errors during sample preparation, injection accuracy/precision and volume changes, various recovery (R.S.) and internal (I.S.) standards were employed (Table 1). Samples and standards were prepared in methylene chloride (GC grade, Fisher Scientific, Pittsburg, PA, USA). The *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA) derivatization agent was obtained from Supelco Analytical (St. Louis, MO, USA).

2.2. Preparation of standard solutions and calibration standards

Standards for method optimization and determination of LODs (compounds marked as *O*, Table 1) were prepared in methylene chloride via serial dilution in the range of 0.15–20 µg/mL for 18 MCAs, 4 MAGs, and 4 DAGs, and in the range of 0.35–50 µg/mL for 18 TAGs. The derivatization protocol of ASTM D 6584 using MSTFA [4] was adapted as follows: 100 µL of MSTFA was added to each of six standard solutions, followed by heating at 60 °C for 1 hour. Butanetriol (1.2 ng) was used as the I.S. for the quantification of acylglycerols. Hexadecanoic acid-*d*₃₁ (0.59 ng) was used as the I.S. for the quantification of MCAs.

Calibration standards (compounds marked as *C*, Table 1) were prepared in methylene chloride at five concentration levels via serial dilution in the range of 2–300 µg/mL for 18 MCAs, 2–160 µg/mL for 4 MAGs, 1.2–100 µg/mL for 4 DAGs, 1.2–40 µg/mL for 6 TAGs, and 0.4–30 µg/mL for glycerol. The concentration ranges of the R.S. (Table 1) in the calibration standards were 3–250 µg/mL for R.S.1a, R.S.1b and R.S.1c, 0.3–25 µg/mL for R.S.2, and 1.5–125 µg/mL for R.S.3 and R.S.4. The standards were derivatized with 100 µL of MSTFA at 60 °C for 1 hour. The final amounts of I.S.1 and I.S.2 were 94 ng and 80 ng, respectively.

A 100-fold molar excess of MSTFA compared to the derivatized species was used to ensure that the derivatization was complete.

2.3. Generation and preparation of OLP samples

Six OLP samples were generated via partial cracking of soybean oil in a miniaturized tubular batch stainless steel reactor (Fig. 1). The reactor body (5 mL) was placed in a heating base with a temperature control system consisting of a solid-state relay (Omega 25A SSR, SSRL240DC25, Omega, Stamford, CT, USA) and a ramp/soak controller (Omega, CN7823). Heating was accomplished using a McMaster-Carr (Robbinsville, NJ, USA) 400 W cartridge heater (3618K454, diameter of 0.371" and voltage of 120 V). An Omega miniature type K ceramic thermocouple probe was used to measure the temperature inside of the reactor during the experiments (SICSS-062U-5-SHX, 0.062" O.D., 5" long). The pressure was monitored by a Swagelok stainless steel miniature industrial pressure gauge (PGI-40M-PG3000-LANX with a working pressure range up to 20.6 MPa, Swagelok, Bismarck, ND, USA). To prevent buildup of pressure, a Swagelok one-piece instrumentation stainless steel ball valve (SS-41S2) rated for 17.2 MPa was used. A high temperature HIP valve (60-11HF2-HT, Erie, PA, USA) rated for 413.7 MPa at 538 °C was used as an outlet valve to enable transfer of the OLP into two sequential collection vials. Swagelok stainless steel fittings and tubing (0.0225" I.D.) were used for all connections.

For OLP generation, the soybean oil (1 mL) was introduced to the reactor, which was subsequently purged with nitrogen. The system was sealed and then heated until the required temperature (470–620 °C) was reached while the pressure was monitored (2.9–10 MPa). The system was held at the desired temperature for 5 min. Upon completion of the experiment (total ca. 10–15 min), the reactor was cooled to near room temperature using a cooling system integrated into the same base containing the heating elements. The OLP samples were transferred into cooled collection vials.

An aliquot (10 µL) of an OLP sample was first mixed with 200 ng of each R.S.1a, R.S.1b, and R.S.1c, 20 ng of R.S.2, and 100 ng of each R.S.3 and R.S.4 (Table 1). The resulting mixture was then derivatized the same way as the calibration standards (described in Section 2.2).

2.4. HTGC-FID/MS analysis

The analyses were performed using an Agilent 7890 GC-FID/5975C MS (Agilent Technologies, Santa Clara, CA, USA) equipped with an autosampler (7386B series) and PTV with

septum (Gerstel, Linthicum Heights, MD, USA) employing a baffled glass liner (Restek, Bellefonte, PA, USA).

To ensure efficient transfer of analytes from the PTV injection port to the column, three injection parameters were optimized in five levels using a CCD (Suppl. Table 2). The first parameter was the hold time (0.70–2.44 min) of the PTV injector at 40 °C. The second was the column hold time (1.09–4.11 min) at 35 °C. The last was the splitless time (1.09–2.60 min). The starting inlet temperature (40 °C) was the minimum temperature that could be reached in a reasonable period of time (i.e., 15 min) without applying cryogenic cooling. The final PTV temperature (380 °C) was set to match the final column temperature. The heating rate (720 °C/min) was set to the maximum possible value to insure complete vaporization of all high molecular mass analytes. A lower PTV heating rate (50 °C/min) was not considered as it was shown to be ineffective in our previous work addressing the analyses of a wide range of alkanes [54]. The optimized injection program applied to the OLP samples was as follows: 40 °C for 0.7 min, 720 °C/min to 380 °C, and hold for 10 min, with a splitless time of 2.6 min.

The chromatographic separation was carried out on a DB-1HT column (15 m x 0.25 mm I.D. x 0.1 µm film thickness) obtained from Agilent Technologies. The injection volume was 0.2 µL. Helium was used as a carrier gas at a constant flow rate of 1 mL/min. The final column temperature program started at 35 °C for 2.6 min followed by ramp #1 (15 °C/min) to 230 °C, ramp #2 (20 °C/min) to 380 °C, and a final hold for 10 min. Total run time was 33 min. This program was shorter than that specified in ASTM D 6584 [4] and accounted for instrument capabilities and separation of more volatile MCAs.

Compounds eluting from the GC column were partitioned via a 2-way splitter with makeup gas (He at a constant pressure of 28 kPa) between the FID and MS detectors in a 2:1 ratio (FID:MS) to provide simultaneous mass spectra identification and FID quantification. The lengths of the connecting capillaries (0.15 mm I.D.) to the MS and FID detectors were 1.71 m and 0.31 m, respectively. FID and MS transfer line temperatures were set to 400 °C and 300 °C, respectively.

The effect of several electron ionization (EI) source / quadrupole analyzer temperatures, namely 350/200 °C, 300/200 °C and 250/150 °C, on the MS fragmentation pattern of TAGs was evaluated. The optimum conditions, were found at 350/200 °C (see Section 3.2). Total ion current (TIC), selected ion monitoring (SIM), and SITI (simultaneous SIM and TIC) quadrupole scan modes were compared. TIC was essential for analyte identification, while SIM was more sensitive. The final sample acquisition was performed in SITI mode since too many ions were required to be monitored at the same time due to the complexity of OLP samples. The quantification and confirmation ions for the target analytes are listed in Table 1. TIC data were acquired in three mass ranges: m/z 50–450 for MCAs, m/z 250–700 for MAGs and DAGs, and m/z 350–750 for TAGs. For the majority of compounds two ions (Table 1) with dwell times of 50 msec were monitored in SIM analysis. Analytes which eluted closer than 0.2 min from each other were combined in one group of four ions and monitored with a 30 msec dwell time. All TAGs were monitored as one group of 14 ions with a 20 msec dwell time. The setup of SITI combined from SIM and TIC scans resulted in nine cycles/peak for most analytes.

A retention time shift of 0.01–0.02 min to earlier elution times was observed for all target analytes every 20–35 sample injections. This may be due to stationary phase modification due to the covering of active sites in the beginning of the column by the sample matrix.

Based on a routine check of the calibration standard responses, we noticed a signal reduction leading to irreproducibility of the MS signal over time, necessitating source cleanup after approximately 50 OLP sample runs (the standards did not seem to have an impact on MS signal reproducibility).

2.5. Identification and quantification

The target species in the OLP samples were identified by matching retention times and mass spectra of analytes to those of standards and in the MS library. To identify heterogeneous acylglycerols, for which neither standards nor NIST spectra were available, we used specific fragmentation ions reported elsewhere [35, 55, 56] and further confirmed in our study. To determine the nature of the fatty acyl chains, the following fragment ions were used: (1) MAGs, $[M-CH_2O-TMS]^+$ fragment ions, (2) DAGs, $[M-RCOO-CH_2]^+$ ions, (3) TAGs, $[RCO+128]^+$ ions (corresponding to one acyl chain attached to a glycerol backbone plus a C_2H_3COO fragment). The identity of acylglycerols was further confirmed by using the fragments with the highest m/z , namely $[M-RCOO]^+$ (i.e., molecular ion minus one fatty acid) in the spectra of TAGs and the $[M-CH_3]^+$ in the spectra of MAGs and DAGs. Positional isomers of DAGs were distinguished by the presence/absence of the $[M-RCOO-CH_2]^+$ ion, which indicated the primary positions (*sn-1/sn-3*) of the acyl groups, whereas an abundant $[M-RCOO]^+$ ion identified the *sn-2* position on the glycerol backbone [56]. To simplify the identification of acylglycerols, we generated Suppl. Table 2 which summarizes fragment ions of all theoretically possible MAGs, DAGs, and TAGs constructed from C_{16} , C_{18} , $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ acid moieties.

For standards, the FID calibration parameters for co-eluting diolein/dilinolein and triolein/trilinolein were calculated from a calibration slope determined by separate analysis of individual compounds. Heterogeneous DAG and TAG standards (i.e., consisting of non-identical acyl chains) were not commercially available, so the quantification of OLP samples was performed based on the prevailing acyl chain identified by MS. FID quantification of DAGs with an acyl carbon number (i.e., total carbon atoms; ACN) of 34 (C_{16} and C_{18}) or 36 (two C_{18}) were performed using dipalmitin and dilinolein, respectively. FID quantification of TAGs with an ACN of 50 (two C_{16} and one C_{18}) was based on tripalmitin calibration. TAGs with an ACN of 52 (two C_{18} and one C_{16}) and with an ACN of 54 (three C_{18}) were quantified using trilinolein calibration. These ACN are the most common for TAGs occurring in biofuel intermediate products as a result of soybean oil noncatalytic cracking.

For MS/TIC quantification of target compounds, the ions listed in Table 1, were employed. For TAGs, quantification methods using two fragmentation ions, namely $[RCO+128]^+$ and $[M-RCOO]^+$, were compared. The $[M-RCOO]^+$ fragments could not be employed for quantifications because of the interference from too many other different ions eluting within one retention time window. The quantification of heterogeneous $[M-RCOO]^+$ ions, i.e., the ions consisting of one C_{16} and one C_{18} acyl chain (observed in TAGs with an ACN of 50 and 52), was performed using the calibration of tripalmitin. Heterogeneous $[M-RCOO]^+$ ions consisting of two different C_{18} chains (observed in TAGs with an ACN of 52 and 54) were quantified using the nearest homogeneous standard ion of higher m/z . Thus, for the quantification using m/z 605 ion (i.e., consisting of stearin and olein acyl chains, Suppl. Table 3), the standard ion of m/z 607 (i.e., consisting of two stearin acyl chains, Suppl. Table 3) was used. The SIM quantification of TAGs was performed using $[RCO+128]^+$ ions only.

To evaluate the matrix's impact on analysis performance, a mid-level calibration solution was analyzed after every three samples and the calibration standards were analyzed after every six sample runs. .

2.6. Data processing and method validation

The GC data were processed via MSD ChemStation E.01.00.237 from Agilent Technologies. CCD experiments were modeled using Minitab® 15.1.30.0 (Minitab Inc., State College, PA, USA). The CCD experiments were performed in duplicate (the central points in quadruplicate) and were split into two blocks, eliminating any potential batch-to-batch source of variability. This resulted in a total of 40 (i.e., 20×2) experiments (Suppl. Table 2). The analysis of variance (ANOVA) and P -

values were calculated to indicate the impact of studied factors and interactions on analyte peak areas. A factor or interaction was considered to be influential if the P -value ≤ 0.05 .

The LODs were calculated from the four lowest concentrations (covering one order of magnitude) of calibration curves using the formula $LOD = 3.3 \cdot s_y / \text{slope}$, where s_y is the standard error for the y estimate [57]. Given a split of gas flow between FID and MS in a ratio of 2:1 (FID:MS), the calculated FID and MS LODs were adjusted by factors of 2/3 and 1/3, respectively. The limits of quantification (LOQ) were calculated as $10 \cdot s_y / \text{slope}$ [57].

Method repeatability was evaluated using six OLP samples. The precision of sample preparation was based on derivatization of triplicate samples. The precision of the chromatographic method was determined by three injections of an OLP sample in random order within the same day (the intra-day repeatability). For inter-day repeatability, nine replicates of OLP samples were analyzed over an eight-day period. Standard deviation (SD) was used to express the precision of the analyses.

The stability of MSTFA-derivatized samples, kept at an ambient temperature (20–25 °C), was determined by injecting the same OLP sample consecutively for up to eight days.

3. Results and discussion

3.1. Optimization of PTV injection conditions

To evaluate the significance of the injection techniques on analyte discrimination, we assessed and optimized three major injection parameters, namely the PTV hold time at an initial 40 °C, the splitless time and the initial oven hold time at 35 °C, using CCD (Suppl. Table 2).

A regression analysis (i.e., P -values in Table 2) of the injection program revealed that the PTV hold time, the splitless time, and the interaction between these two parameters had a statistically significant effect on the response of the target analytes, particularly of the high MW species. On the other hand, the following parameters did not significantly affect the response of a majority of the analytes: the time for which the column was kept at an initial temperature, interaction between the column and the PTV hold times at the initial temperature, column hold time and splitless time. This trend was observed even for experiments where the column time at an initial temperature was shorter than that of the PTV hold time.

PTV hold times below 1.5 min at an initial temperature of 40 °C and splitless times longer than 2.5 min resulted in a higher response for most of the analytes (Fig. 2) except for volatile low MW MCAs, namely C₄, C₆ and C₇ (Table 2). Moreover, C₄–C₆ MCAs were effectively transferred from the PTV to the column in a splitless time of only one min. Splitless times longer than 2.5 min decreased the separation of butyric acid and the derivatization agent.

Interaction between the PTV hold time at an initial temperature and the splitless time was significant only for high MW species (Table 2). The representative interaction patterns are displayed in Fig. 3 as response surface plots (RSP) for C₈ MCA and C₁₆ TAG. In the analyses where the splitless time was lower than the PTV time, the response of a majority of the analytes was at a minimum; no signal was observed for C₁₈–C₂₂ TAGs (Fig. 4). As demonstrated in Fig. 4, the optimal conditions were obtained whenever the difference between splitless and PTV times was one minute or higher thus allowing for complete transfer of compounds heavier than C₇.

The optimum PTV injection conditions and chosen values for the analysis of OLP samples are listed in the Table 2. Since the time during which the column stays at the initial 35 °C was influential, it was set to be equal to the splitless time, for consistency reasons.

3.2. Effect of EI source temperature

The fragmentation patterns in mass spectra are typically affected by the temperature of the ionization source. While lower temperatures may be beneficial in preserving the high mass ions, higher temperatures may eliminate a number of background ions (i.e., providing cleaner mass spectra)

[58]. Various EI source temperatures for the analysis of TAGs have been reported in the literature, ranging from 175 [36] to 370 °C [34]. However to our knowledge, no experimental evaluation of the most efficient ionization conditions has been previously reported.

Our results showed that the temperature of the EI source mainly affected the apparent concentration of TAGs. Among the three ion source temperatures tested, 250 °C, 300 °C and 350 °C, 350 °C provided the highest response of $[M-RCOO]^+$ fragment in C₁₄ TAG, C₁₆ TAG, and C₁₈ TAG mass spectra (Fig. 5a). The signal-to-noise ratio (S/N) for these types of fragments could not be evaluated as no noise was observed. For the $[RCO+128]^+$ fragment, the S/N values were adjusted for the root mean square (RMS) value of the measured noise and expressed as RMS S/N. The highest RMS S/N was observed for EI source temperatures of 300 °C (C₁₄ and C₁₆ TAGs, Fig. 5b) and 350 °C (C₁₈ TAG, Fig. 5b), respectively. Since $[M-RCOO]^+$ high mass fragments are more suitable for the identification of TAGs, the highest ion source temperature, 350 °C, was chosen for all further experiments. The temperature of the quadrupole analyzer was adjusted only to reduce the temperature gradient between the source and the analyzer.

3.3. Separation of MCAs, MAGs, DAGs, TAGs and glycerol

Several oven temperature programs were evaluated in order to achieve sufficient separation of target analytes. The temperature program proposed by ASTM D 6584 [4] (i.e., initial temperature 50 °C, hold 1 min, gradient #1 = 15 °C /min to 180 °C, gradient #2 = 7 °C /min to 230 °C, gradient #3 = 30 °C /min to 380 °C, hold for 10 min) was found to be unsuitable for the latest model of Agilent 7890A GC used in this study. Since the maximum applicable temperature gradient with a reproducible heating rate for the Agilent 7890A GC is 20 °C/min for temperatures above 300 °C, gradient #3 from ASTM D 6584 [4] was decreased accordingly. Also, it was found that the slow gradient #2 (7 °C /min) in the ASTM method did not improve the resolution between unsaturated MAGs, and thus it was combined with gradient #1 of 15 °C /min. Further increase in the ramp rate for gradient #1 (up to gradient #3 of 20 °C /min, which would allow the use of a single temperature gradient) resulted in lower resolution between unsaturated C₁₈ MCAs. The final temperature program was: initial temperature of 35 °C for 2.6 min followed by gradient #1 with a ramp rate of 15 °C/min to 230 °C, gradient #2 with a ramp rate of 20 °C/min to 380 °C, and a final hold for 10 min. a four min faster The total analysis time was decreased by 4 minutes compared to ASTM D 6584 [4], and provided a more stable linear temperature increase.

The temperature program we developed resulted in a separation of major classes of compounds (Fig. 6), as required in ASTM D 6584 [4]. In addition, otherwise co-eluting monoolein and monolinolein were sufficiently resolved ($R_s=0.6$) to enable their quantification. We were also able to separate positional isomers of MAGs and DAGs (i.e., *sn*-2 or *sn*-1,3 acyl chain positions on glycerol backbone). Unsaturated DAGs were baseline separated from distearin, with diolein overlapping with dilinolein. Separation of TAGs was similar to the ASTM method, i.e., tristearin co-eluted with trilinolenin, and triolein overlapped with trilinolein.

3.4. Calibration parameters and limits of detections

Calibration parameters for all target analytes and for all monitored signals (i.e., FID, TIC, TIC/EIC, SIM) are summarized in Suppl. Tables 4 and 5.

The FID signal provided similar slopes for saturated C₅–C₁₀ MCAs (0.0168 ± 0.0007) and for C₁₁–C₁₈ MCAs (0.0214 ± 0.0006), with slightly lower sensitivity observed for unsaturated C₁₈ MCAs (Suppl. Table 4). Butyric acid partially co-eluted with R.S.1a (Fig.6) which led to its lower sensitivity. Similar FID signal slopes were observed for all saturated and unsaturated MAGs and DAGs (0.0210 ± 0.0008 and 0.0193 ± 0.0008 , respectively, Suppl. Table 5). By contrast, the sensitivity for TAGs decreased with an increasing acyl chain length and with the number of double bonds.

ASTM D 6584, which is designed for biodiesel, recommends using the FID slope of C_{18:1} acylglycerols (i.e., monoolein, diolein and triolein) for quantification of all other acylglycerols in series [4]. As shown above, this assumption was only appropriate for MAGs and DAGs. Based on our results, the application of ASTM D 6584 may lead to an underestimate of the TAGs because the slopes for trilinolein and trilinolenin were 1.5 and 2 times, respectively, lower than that of triolein (Suppl. Table 5). Therefore, the use of the triolein slope to determine the concentrations of polyunsaturated TAGs with prevailing C_{18:2} and C_{18:3} acyl chains may lead to underestimation of the results depending on sample type.

The MS signal (TIC, TIC/EIC, or SIM) produced a high slope dependency on acyl chain length and degree of unsaturation in both MCAs and acylglycerols (Suppl. Tables 4 and 5, respectively). These results, obtained for homogenous (i.e., containing identical acids chains) TAGs, were in agreement with results reported by other research groups [36, 37, 41] for a series of homogeneous and heterogeneous TAGs. Although lower sensitivity was observed for the MS signal, quantification of acylglycerols using the fragmentation ions listed in Table 1 can provide quantitative information on the distribution of acyl chains in heterogeneous species, as discussed in section 3.5.2.

The instrumental LOD values for targeted MSTFA-derivatized compounds obtained using FID and MS (using [RCO+128]⁺ fragments for TAGs) signals are listed in Table 3. As mentioned in Section 2.5, TAG quantification using [M-RCOO]⁺ ions provided higher variability. The LODs (not shown) in MS/TIC mode were in a range of 0.2–1.3 ng. It was not feasible to evaluate TAG LODs using [M-RCOO]⁺ ions in MS/SIM mode as too many ions would have to be monitored.

As expected, the lowest LODs were obtained in a SIM mode resulting in 0.01–0.05 ng for MCAs and 0.03–0.14 ng for acylglycerols. FID, which is considered to be a nearly universal detector, provided LODs of 0.07–0.27 ng for both MCAs and acylglycerols. Higher LODs were observed for trilinolein and trilinolenin, due to the high degree of unsaturation, and for butanoic acid, which eluted near to the MSTFA peak. For TIC/EIC signal LOD values increased with an increase of the acyl chain length and degree of unsaturation, ranging from 0.03 to 0.18 ng for MCAs, and 0.03 to 15.5 ng for acylglycerols (Table 3). For acids the determined LODs were higher than those reported previously [59] for analysis of MCA possibly due to a narrower *m/z* range used previously.

In general, for all of the signals (i.e., FID, TIC/EIC, and SIM), we were able to obtain LOD values below the working range (6–57 ng) suggested for the analysis of acylglycerols in ASTM D 6584 [4]. The only exception was for the LOD value of trilinolenin (i.e., 15.5 ng) obtained using TIC/EIC mode. However this compound was not used in the ASTM method at all.

3.5. Characterization of OLPs

The HTGC-FID/MS method we developed was applied to six OLP samples generated by the partial cracking of soybean oil. The samples were prepared under different temperatures representing various degrees of noncatalytic thermal cracking. These pyrolysis experiments were performed at relatively short residence times, 5–10 min, to yield a high concentration of DAGs and TAGs. While these conditions are not commercially attractive, they were beneficial for analytical method evaluation. A detailed evaluation of reproducibility was completed for two representative samples (Section 3.6) using FID and MS (Tables 4 and 5, respectively). The described trends were confirmed with the FID and MS data for the other four soybean oil OLP samples (Suppl. Tables 6 and 7).

The chromatographic resolution of OLP compounds was based on their total number of carbon atoms, positional isomerization, and, to some extent, the degree of unsaturation (Fig. 7). Satisfactory sensitivity of the developed HTGC-FID/MS method and the absence of significant interferences from the oil matrix were demonstrated. The only complications observed were for co-eluted heterogeneous TAGs, resulting in equivocal peak identification. The quantification of target species was successfully performed by both FID and MS signals as discussed below.

3.5.1. MCAs analysis in the OLPs

The total organic acid concentration in the soybean OLP samples ranged from 5.4 to 13.0 weight % (Tables 4 and 5, Suppl. Table 6). The MCA composition varied slightly from sample to sample, with oleic, linoleic and palmitic acids being the most predominant (in equivalent amounts, Table 5). The detected high concentrations of heptanoic acid demonstrated the necessity to monitor MCAs shorter than C₁₆ and C₁₈. The relatively high concentrations of C₅–C₁₀ MCA observed in the study were in agreement with previously reported results for OLPs obtained by non-catalytic cracking [60].

C₁₁ MCA was only detected by FID in the soybean OLP samples, even though the C₁₁ MCA LOD in the FID signal was higher than in MS/SIM for the standards (Table 3). The MS spectra of the C₁₁ MCA peak revealed a contribution of unsaturated C_{11:1} MCA, which resulted in an overestimation of the C₁₁ MCA concentration by FID.

Although the baseline separation of the critical pair of oleic and linoleic acids was obtained in the standard mixture (Fig. 6), in soybean oil OLP samples C_{18:2} MCA was present in the form of three isomeric peaks, resulting in partial co-elution with C_{18:1} MCA (detected as two resolved isomers of equal abundance). Should the detailed composition of unsaturated C₁₈ MCAs be required, quantification by MS signal may be preferred (both TIC/EIC and SIM produced the same results). However, the total amounts of MCAs were similar and thus may be estimated by either FID or MS (Tables 4 and 5).

3.5.2. Acylglycerol analysis in the OLPs

The novelty of this work centers around an evaluation of the feasibility of TIC/EIC for acylglycerol speciation and quantification, and its comparison to common FID approaches applied to OLP samples. The MS SIM is not recommended for quantification due to the complexity of the OLP samples, and the consecutive requirement to simultaneously monitor too many ions. Quantification of acylglycerols using MS and FID provided similar results (Table 4 and 5, Suppl. Table 6), demonstrating the comparable accuracy of both methods. Most discrepancies were attributed to higher LODs for MS EIC detection, though some can be related to the lack of speciation using FID (as shown below).

The majority of the detected MAGs were below LOQ levels. Only monolinolein was observed when employing FID. While its presence was confirmed in SIM, in MS/TIC analysis it was below LOQ (Table 5).

DAGs with ACNs of 34 and 36 were baseline resolved, each into two peaks (Fig. 7), indicating the presence of two structural isomers, i.e., 1,3-DAGs 1,2-DAGs. The MS revealed the heterogeneous nature of DAGs with an ACN of 36, with a C_{18:2} acyl chain concentration being much higher than that of C_{18:1} and C_{18:3} acyl chains. The total concentrations quantified by MS were lower than those obtained by FID as some of the DAGs could not be detected by FID due to the higher LODs.

TAGs in soybean oil OLPs were resolved into three major peaks according to their carbon number (Fig. 7). The dominant components were unsaturated (up to 6 double bonds) TAGs with ACN of 54, i.e., (containing three C₁₈ acyl chains), TAGs with an ACN of 52 (containing two C₁₈ acyl chains and one C₁₆ acyl chain) and the most abundant TAGs with an ACN 50 (two C₁₆ acyl chains and one C₁₈ acyl chain). Similar results for FID and MS were obtained for TAGs with an ACN of 52. Higher concentrations of TAGs with an ACN of 54 obtained by FID (compared to MS) may be attributed to the selection of a single FID calibration standard for all levels of saturation. This approach could have resulted in an overestimation of TAGs having a higher degree of saturation than trilinolein.

3.6. Method repeatability

The repeatability of the sample derivatization protocol and intra- and inter-day HTGC analysis for the target compounds in soybean oil OLPs using FID and MS are summarized in Tables 4 and 5, respectively. The lowest standard deviations (SDs) were observed for intra-day GC analysis for both FID and MS signals. For the inter-day analysis, the instrumental variability increased up to 3-fold for 8-day old samples. Some differences in the results were caused by an increase in the LOQ values of 8-day old standards, particularly for the TAG $[RCO+128]^+$ fragmentation ions. However, based on the ratios of peak area to area of internal standard, the MSTFA-derivatized samples seemed to be stable after eight days of storage at laboratory room temperature (ca. 17 °C). Sample preparation (i.e., derivatization) exhibited the highest degree of variability with up to 10 % RSD.

4. Conclusions

In this work, we demonstrated the applicability of the HTGC-FID/MS method for the simultaneous identification and quantification of a variety of TAG decomposition products. To our knowledge, it is the first successful simultaneous analysis of MCAs, MAGs, DAGs, and TAGs from a single run.

Optimization of the PTV injection conditions, column temperature program, and MS parameters allowed for the separation of the majority of the target compounds based on their total number of carbon atoms, positional isomerization and, to some extent, degree of unsaturation. The optimum PTV conditions were particularly critical for detection of TAGs. The application of MS detection offered complementary information to the FID on the structure of co-eluting analytes and enabled more accurate quantification than FID alone. Results from the MS speciation of heterogeneous TAGs were used to select the most appropriate FID quantification standard.

In this paper, the suggested quantification method using TIC/EIC provided similar results to those obtained by FID, thus the developed method can solely employ an MS detector. However, MS requires more frequent cleanup and its sensitivity is affected by relatively high bleed from the column. Routine analysis of standards before and after oil or biofuel samples is an important step for ensuring high accuracy analysis. It is also important to note that similar to ASTM method D 6584, we have used fairly diluted samples [4]. The detection limits could be further improved if the analyzed samples were more concentrated. However, this would be detrimental to the detection of butanoic acid as it would elute in an extended solvent delay necessary due to higher volume of derivatization agent used (or its products).

This method, together with our previous work on the quantification of the hydrocarbon content of biofuels [61], has the potential to be incorporated into standard methods used by the emerging next generation biofuels industry since they both provide detailed and complete characterization of the major by-products of biofuel production, with a higher degree of accuracy for both identification and quantification than currently applied standard methods.

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Figure Captions

Fig. 1. Batch reactor used for the noncatalytic cracking of soybean oil.

Fig. 2. CCD main effect plots of (A, C) the PTV hold time at an initial temperature of 40 °C and (B, D) the splitless time as functions of a peak area for MCAs (A, B) and selected TAGs (C, D). MCAs C₁₁-C₁₈ followed analogous to MCA C₁₀ trends.

Fig. 3. Response surface plots for the PTV hold time at an initial temperature of 40 °C and splitless time obtained via central composite design for (A) MCA C₈ (MCAs C₉-C₁₈ followed analogous trend) and (B) TAG C₁₆ (TAGs C₈-C₁₅ and TAGs C₁₇-C₂₂ showed analogous trend). The oven hold time at an initial temperature of 35 °C was kept at 2.6 min.

Fig. 4. Difference between the splitless time and the PTV hold time at an initial temperature of 40 °C as a function of a peak area for (A) MCA C₁₁-C₁₈ and (B) representative TAGs.

Fig. 5. The dependence of (A) signal height (ion [M-RCOO]⁺, no noise was observed), (B) signal to noise ratio (RMS S/N) (ion [RCO+128]⁺) of representative TAGs on EI source / quadrupole analyzer temperatures, obtained in TIC/EIC mode.

Fig. 6. HTGC-FID chromatogram of standard mixture of MCAs, MAGs, DAGs, TAGs and glycerol after derivatization with MSTFA. Peak identification is reported in Table 1.

Fig. 7. (A) HTGC-FID chromatogram and (B) HTGC-MS/TIC chromatogram of OLP 2 (see Tables 4 and 5 for detailed composition) obtained simultaneously after derivatization with MSTFA. Recovery and internal standards are reported in Table 1.

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709

Figure 1

Figure 1

Figure 2

Figure 2

Figure 3

Figure 3

Figure 4

Figure 4

Figure 5

Figure 5

Figure 6

Figure 6

Figure 7

Figure 7

(A) FID

(B) MS/TIC

Table 1

Table 1. Reagents used: (1) for method optimization (O) and (2) as calibration standards (C), including recovery and internal standards (R.S. and I.S., respectively). Molecular weights and target ions are provided for MSTFA derivatized compounds.

Purpose ^a	Name	Supplier ^b	Abbreviation	Formula	TMS-derivatives MW (g/mol)	Quantitation ion (symbol)	Confirmation ion (symbol)
	Monocarboxylic acids		MCAs			[M-CH₃]⁺	[TMS+COO]⁺
C	Butanoic acid	Sigma-Aldrich	C ₄ MCA	C ₃ H ₇ COOH	160	145	117
O, C	Pentanoic acid	Sigma-Aldrich	C ₅ MCA	C ₄ H ₉ COOH	174	159	117
O, C	Hexanoic acid	Acros	C ₆ MCA	C ₅ H ₁₁ COOH	188	173	117
O, C	Heptanoic acid	Acros	C ₇ MCA	C ₆ H ₁₃ COOH	202	187	117
O, C	Octanoic acid	Acros	C ₈ MCA	C ₇ H ₁₅ COOH	216	201	117
O, C	Nonanoic acid	MP Biomedicals	C ₉ MCA	C ₈ H ₁₇ COOH	230	215	117
O, C	Decanoic acid	Acros	C ₁₀ MCA	C ₉ H ₁₉ COOH	244	229	117
O, C	Undecanoic	Acros	C ₁₁ MCA	C ₁₀ H ₂₁ COOH	258	243	117
O, C	Dodecanoic	Sigma-Aldrich	C ₁₂ MCA	C ₁₁ H ₂₃ COOH	272	257	117
O, C	Tridecanoic	MP Biomedicals	C ₁₃ MCA	C ₁₂ H ₂₅ COOH	286	271	117
O, C	Tetradecanoic	Alfa Aesar	C ₁₄ MCA	C ₁₃ H ₂₇ COOH	300	285	117
O, C	Pentadecanoic	Acros	C ₁₅ MCA	C ₁₄ H ₂₉ COOH	314	299	117
O, C	Hexadecanoic	Acros	C ₁₆ MCA	C ₁₅ H ₃₁ COOH	328	313	117
O, C	Heptadecanoic	Alfa Aesar	C ₁₇ MCA	C ₁₆ H ₃₃ COOH	342	327	117
O, C	Octadecanoic	Acros	C ₁₈ MCA	C ₁₇ H ₃₅ COOH	356	341	117
O, C	Oleic acid	Sigma-Aldrich	MCA C _{18:1}	C ₁₇ H ₃₃ COOH	354	339	117
C	Linoleic acid	Sigma-Aldrich	MCA C _{18:2}	C ₁₇ H ₃₁ COOH	352	337	117
C	α -Linolenic	Sigma-Aldrich	MCA C _{18:3}	C ₁₇ H ₂₉ COOH	350	335	117
R.S. 1a	Butanoic acid- <i>d</i> ₇	CDN Isotopes	C ₄ - <i>d</i> ₇ MCA	C ₃ D ₇ COOH	168	152	
R.S. 1b	Nonanoic acid- <i>d</i> ₁₇	CDN Isotopes	C ₈ - <i>d</i> ₁₇ MCA	C ₈ D ₁₇ COOH	248	232	117
R.S. 1c	Hexadecanoic acid- <i>d</i> ₃₁	CDN Isotopes	C ₁₆ - <i>d</i> ₃₁ MCA	C ₁₅ D ₃₁ COOH	360	344	120
I.S.1	2-Chlorotoluene	Sigma-Aldrich	2-chloro-toluene	C ₇ H ₇ Cl	127	91	126
	Alcohols		AOLs				
R.S. 2	Butanetriol	Sigma-Aldrich	butanetriol	C ₄ H ₁₀ O ₃	272	219	
C	Glycerol	Sigma-Aldrich	glycerol	C ₃ H ₅ (OH) ₃	309	205	147
R.S. 3	<i>n</i> -Eicosanyl- <i>d</i> ₄₁ alcohol	CDN Isotopes	C ₂₀ - <i>d</i> ₄₁ AOL	C ₂₀ D ₄₁ OH	412	396	105
	Monoacylglycerols		MAGs			[M-CH₂O-TMS]⁺	[M-CH₃]⁺
O, C	Monopalmitin	Sigma-Aldrich	C ₁₆ MAG	C ₁₉ H ₄₀ O ₄	474	371 (P)	459
O, C	Monostearin	Sigma-Aldrich	C _{18:0} MAG	C ₂₁ H ₅₂ O ₄	502	399 (S)	487
O, C	Monoolein	Sigma-Aldrich	C _{18:1} MAG	C ₂₁ H ₅₀ O ₄	500	397 (O)	485
O, C	Monolinolein	Sigma-Aldrich	C _{18:2} MAG	C ₂₁ H ₄₈ O ₄	498	395 (L)	483

Purpose ^a	Name	Supplier ^b	Abbreviation	Formula	TMS-derivatives MW (g/mol)	Quantitation ion (symbol)	Confirmation ion (symbol)
	Diacyl-glycerols		DAGs			[M-RCOO-CH ₂] ⁺	[M-CH ₃] ⁺
O, C	Dipalmitate	Nu-Chek Prep.	C ₁₆ DAG	C ₃₅ H ₆₈ O ₅	640	371 (P)	625
C	Distearin	Sigma-Aldrich	C ₁₈ DAG	C ₃₉ H ₇₆ O ₅	696	399 (S)	681
C	Diolein	Sigma-Aldrich	C _{18:1} DAG	C ₃₉ H ₇₂ O ₅	692	397 (O)	677
O, C	Dilinolein	Sigma-Aldrich	C _{18:2} DAG	C ₃₉ H ₆₈ O ₅	688	395 (L)	673
	Triacyl-glycerols		TAGs			[RCO+128] ⁺	[M-RCOO] ⁺
I.S.2	Tridecanoin	Sigma-Aldrich	C ₁₀ TAG	C ₃₃ H ₆₂ O ₆	555	283	383
C	Tripalmitin	GLC-437, Nu-Chek Prep.	C ₁₆ TAG	C ₅₁ H ₉₈ O ₆	807	367 (P)	551 (PP)
C	Tristearin	GLC-437, Nu-Chek Prep.	C ₁₈ TAG	C ₅₇ H ₁₁₀ O ₆	891	395 (S)	607 (SS)
C	Triolein	GLC-437, Nu-Chek Prep.	C _{18:1} TAG	C ₅₇ H ₁₀₄ O ₆	885	393 (O)	603 (OO)
C	Trilinolein	GLC-437, Nu-Chek Prep.	C _{18:2} TAG	C ₅₇ H ₉₈ O ₆	879	391 (L)	599 (LL)
C	Trilinolenin	GLC-437, Nu-Chek Prep.	C _{18:3} TAG	C ₅₇ H ₉₂ O ₆	873	389 (Ln)	595 (LnLn)
C	Triarachidin	GLC-437, Nu-Chek Prep.	C ₂₀ TAG	C ₆₃ H ₁₂₂ O ₆	975	423	663
O	Trioctanoin	GLC-768, Nu-Chek Prep.	C ₈ TAG	C ₂₇ H ₅₀ O ₆	471	255	327
O	Trinonanoin	GLC-768, Nu-Chek Prep.	C ₉ TAG	C ₃₀ H ₅₆ O ₆	513	269	355
O	Tridecanoin	GLC-768, Nu-Chek Prep.	C ₁₀ TAG	C ₃₃ H ₆₂ O ₆	555	283	383
O	Triundecanoin	GLC-768, Nu-Chek Prep.	C ₁₁ TAG	C ₃₆ H ₆₈ O ₆	597	297	411
O	Trilaurin	GLC-768, Nu-Chek Prep.	C ₁₂ TAG	C ₃₉ H ₇₄ O ₆	639	311	439
O	Tritridecanoin	GLC-768, Nu-Chek Prep.	C ₁₃ TAG	C ₄₂ H ₈₀ O ₆	681	325	467
O	Trimyristin	GLC-768, Nu-Chek Prep.	C ₁₄ TAG	C ₄₅ H ₈₆ O ₆	723	339	495
O	Tripentadecanoin	GLC-768, Nu-Chek Prep.	C ₁₅ TAG	C ₄₈ H ₉₂ O ₆	765	353	523
O	Tripalmitin	GLC-768, Nu-Chek Prep.	C ₁₆ TAG	C ₅₁ H ₉₈ O ₆	807	367	551
O	Triheptadecanoin	GLC-768, Nu-Chek Prep.	C ₁₇ TAG	C ₅₄ H ₁₀₄ O ₆	849	381	579
O	Tristearin	GLC-768, Nu-Chek Prep.	C ₁₈ TAG	C ₅₇ H ₁₁₀ O ₆	891	395	607
O	Triolein	Sigma-Aldrich	C _{18:1} TAG	C ₅₇ H ₁₀₄ O ₆	885	393	603
O	Trinonadecanoin	GLC-768, Nu-Chek Prep.	C ₁₉ TAG	C ₆₀ H ₁₁₆ O ₆	933	409	635

O	Triarachidin	GLC-768, Nu-Chek Prep.	C ₂₀ TAG	C ₆₃ H ₁₂₂ O ₆	975	423	663
O	Triheneicosanol	GLC-768, Nu-Chek Prep.	C ₂₁ TAG	C ₆₆ H ₁₂₈ O ₆	1017	437	691
O	Tribehenin	GLC-768, Nu-Chek Prep.	C ₂₂ TAG	C ₆₉ H ₁₃₄ O ₆	1059	451	719
R.S. 4	Tripalmitin- <i>d</i> ₉₈	CDN Isotopes	C ₁₆ - <i>d</i> ₉₈ TAG	C ₅₁ D ₉₈ O ₆	897	406	619

^a Were used for method validation and quantification of samples. R.S.1a was used for MCAs C₄–C₆, R.S.1b - for MCAs C₇–C₁₃, and R.S.1c - for MCAs C₁₄–C₁₈ and unsaturated MCAs C₁₈. R.S.2 was used for glycerol, R.S.3 - for MAGs and DAGs, and R.S.4 - for TAGs. I.S.1 was applied to all MCAs and glycerol, and I.S.2 - to all acylglycerols.

^b Sigma-Aldrich (St. Louis, MO, USA), Acros (Fisher Scientific, Pittsburg, PA, USA), MP Biomedicals (Solon, OH, USA), Alfa Aesar (Ward Hill, MA, USA), CDN Isotopes (Pointe-Claire, Canada), Nu-Chek Prep. Inc. (Elysian, MN, USA).

Table 2

Table 2. *P*-values obtained in central composite design for specific analytes and final optimized PTV injection parameters for the HTGC-FID/MS analysis of MCAs and acylglycerols in soybean oil OLPs.

<i>P</i> -values ^a														
Source	Target compounds													
	MCAs											TAGs		
	C ₄	C ₅	C ₆	C ₇	C ₈	C ₉	C ₁₀	C ₁₅	C ₁₆	C ₁₇	C ₁₈	C ₁₀	C ₁₆	C ₁₈
<i>Main factors</i>														
PTV injection hold time at initial 40 °C	0.4	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	7	0	1	9	1	0	0	0	0	0	0	1	1	3
Splitless time	0.4	0.4	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	2	6	2	0	0	0	0	0	0	0	1	0	0	0
Oven hold time at initial 35 °C	0.1	0.3	0.0	0.5	0.6	0.7	0.3	0.2	0.3	0.3	0.4	0.4	0.4	0.7
	6	9	1	5	5	3	4	4	4	9	0	1	9	7
<i>Interactions</i>														
PTV × Splitless	0.2	0.6	0.9	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	6	7	4	7	0	0	0	0	0	0	0	0	0	0
PTV × Column	0.7	0.7	0.9	0.9	0.9	0.9	0.8	0.5	0.5	0.8	0.6	0.8	0.7	0.6
	3	0	6	8	8	1	7	0	5	3	2	0	8	4
Splitless × Column	0.7	0.6	0.9	0.8	0.9	0.8	0.7	0.7	0.9	0.4	0.3	0.6	0.7	0.8
	3	1	5	7	1	9	9	1	7	9	2	0	4	3
Optimized parameters														
Injection conditions	PTV injection hold time at an initial (40 °C) temperature, min					Splitless time, min					Oven hold time at an initial (35 °C) temperature, min			
	Evaluated					1.09–4.11					1.09–4.11			
Optimum	0.26–1.35					2.6–4.11					1.09–4.11			
Selected	0.7					2.6					2.6			

^a Parameter or interaction highlighted in bold was considered to be influential as *P*-value ≤ 0.05.

Table 3. Instrumental LODs (ng) for target compounds obtained upon derivatization with MSTFA. Analysis was carried out via HTGC-FID/MS, operated in SITI mode with injection volume of 0.2 μ L. Given 2:1 FID to MS split ratio, the LODs were adjusted accordingly. The MS quantitation was performed using ions listed in Table 1.

Target compounds	FID	TIC/EIC (SITI)	SIM/EIC (SITI)
Pentanoic acid	0.09	0.05	0.02
Hexanoic acid	0.21	0.06	0.01
Heptanoic acid	0.06	0.03	0.03
Octanoic acid	0.11	0.05	0.02
Nonanoic acid	0.17	0.07	0.01
Decanoic acid	0.16	0.06	0.03
Undecanoic acid	0.08	0.03	0.02
Dodecanoic acid	0.11	0.06	0.05
Tridecanoic acid	0.09	0.07	0.03
Tetradecanoic acid	0.10	0.11	0.04
Pentadecanoic acid	0.07	0.06	0.02
Hexadecanoic acid	0.26	0.09	0.02
Heptadecanoic acid	0.11	0.17	0.01
Oleic acid	0.07	0.18	0.02
Octadecanoic acid	0.07	0.05	0.03
1-Monopalmitin	0.27	0.07	0.07
1-Monolinolein	0.25	2.12	0.14
1-Monoolein	0.22	0.38	0.05
1-Monostearin	0.18	0.12	0.11
1,2-Dipalmitin	0.07	0.06	0.02
1,2-Dilinolein	0.11	1.75	0.07
Quantified as [RCO+128] ⁺			
Trioctanoin	0.10	0.03	0.05
Trinonanoin	0.08	0.32	0.05
Tridecanoin	0.09	0.01	0.05
Triundecanoin	0.09	0.09	0.06
Trilaurin	0.11	0.19	0.05
Tritridecanoin	0.09	0.33	0.06
Trimyristin	0.12	0.08	0.06
Tripentadecanoin	0.10	0.15	0.06
Tripalmitin	0.13	0.12	0.06
Triheptadecanoin	0.09	0.16	0.07
Triolein	0.10	1.06	0.07
Tristearin	0.11	1.42	0.06
Trinonadecanoin	0.11	1.21	0.03
Triarachidin	0.15	1.76	0.08

Triheneicosanoin	0.19	1.80	0.12
Tribehenin	0.27	0.32	0.10

Table 4. Sample preparation, intra- and inter-day repeatability of all target analytes in soybean oil organic liquid products (OLPs) quantified by GC/FID. The OLP samples were obtained after 5 min exposure to listed temperature and pressure. The results are presented as mean $\pm$ one standard deviation.

Component	OLP 1 (494 °C, 6205 kPa)			OLP 2 (578 °C, 7584 kPa)		
	Repeatability for					
	Sample preparation (derivatization)	Intra-day (injection)	Inter-day (over 8 days)	Sample preparation (derivatization)	Intra-day (injection)	Inter-day (over 8 days)
Monocarboxylic acids						
C ₄ MCA	<LOQ ^a	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
C ₅ MCA	0.31 ± 0.01	0.29 ± 0.01	0.29 ± 0.02	0.32 ± 0.01	0.32 ± 0.01	0.33 ± 0.01
C ₆ MCA	0.44 ± 0.02	0.44 ± 0.01	0.45 ± 0.03	0.46 ± 0.01	0.47 ± 0.02	0.48 ± 0.01
C ₇ MCA	1.38 ± 0.07	1.50 ± 0.01	1.37 ± 0.07	1.22 ± 0.02	1.25 ± 0.02	1.26 ± 0.02
C ₈ MCA	0.50 ± 0.04	0.55 ± 0.01	0.53 ± 0.02	0.68 ± 0.01	0.69 ± 0.01	0.69 ± 0.01
C ₉ MCA	0.31 ± 0.03	0.37 ± 0.01	0.33 ± 0.02	0.45 ± 0.01	0.45 ± 0.01	0.46 ± 0.01
C ₁₀ MCA	0.70 ± 0.04	0.77 ± 0.01	0.69 ± 0.03	0.62 ± 0.01	0.63 ± 0.01	0.63 ± 0.01
C ₁₁ MCA	0.11 ± 0.02	0.14 ± 0.01	0.13 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	0.15 ± 0.01
C ₁₂ MCA	<LOQ	<LOQ	<LOQ	0.03 ± 0.01	<LOQ	<LOQ
C ₁₃ MCA	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
C ₁₄ MCA	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
C ₁₅ MCA	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
C ₁₆ MCA	2.36 ± 0.10	2.46 ± 0.01	2.27 ± 0.09	1.44 ± 0.02	1.43 ± 0.01	1.44 ± 0.03
C ₁₇ MCA	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
C _{18:2} MCA	1.34 ± 0.06	1.36 ± 0.01	1.29 ± 0.07	0.53 ± 0.01	0.55 ± 0.01	0.53 ± 0.02
C _{18:3} MCA	ND ^b	ND	ND	ND	ND	ND
C _{18:1} MCA	4.97 ± 0.33	5.33 ± 0.07	5.11 ± 0.23	2.37 ± 0.04	2.45 ± 0.03	2.52 ± 0.06
C _{18:0} MCA	0.81 ± 0.03	0.87 ± 0.02	0.84 ± 0.03	0.49 ± 0.01	0.50 ± 0.01	0.50 ± 0.01
MCAs total	12.88 ± 0.67	14.09 ± 0.05	13.30 ± 0.48	8.67 ± 0.11	9.20 ± 0.06	8.99 ± 0.06
Glycerol	ND	ND	ND	ND	ND	ND
Monoacylglycerols						
C ₁₆ MAG	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
C _{18:2} MAG	0.28 ± 0.01	0.24 ± 0.01	0.23 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
C _{18:1} MAG	ND	ND	ND	ND	ND	ND
C _{18:0} MAG	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
MAGs total	0.28 ± 0.01	0.24 ± 0.01	0.23 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
Diacylglycerols						
DAG 34 total	0.06 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
1,2(2,3)-DAG 36	0.19 ± 0.03	0.21 ± 0.01	0.18 ± 0.02	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
1,3-DAG 36	0.33 ± 0.07	0.42 ± 0.01	0.32 ± 0.03	0.07 ± 0.01	0.06 ± 0.01	0.05 ± 0.01
DAGs total	0.58 ± 0.10	0.71 ± 0.01	0.66 ± 0.03	0.11 ± 0.01	0.10 ± 0.01	0.09 ± 0.01
Triacylglycerols						

TAG 50	0.44 ± 0.03	0.45 ± 0.04	0.38 ± 0.06	0.16 ± 0.01	0.16 ± 0.01	0.18 ± 0.01
TAG 52	6.45 ± 0.30	6.13 ± 0.10	5.70 ± 0.41	2.67 ± 0.06	2.35 ± 0.06	2.27 ± 0.07
TAG 54	14.47 ± 1.18	13.31 ± 0.12	11.71 ± 1.12	5.63 ± 0.16	4.92 ± 0.10	4.52 ± 0.20
TAGs total	21.36 ± 1.50	19.89 ± 0.23	17.79 ± 1.52	8.46 ± 0.25	7.43 ± 0.26	6.97 ± 0.26

^aBelow the limit of quantification, ^bNot detected.

Table 5. Sample preparation, intra- and inter-day repeatability of all target analytes in soybean oil organic liquid products (OLPs) TIC/EIC. The OLP samples were obtained after 5 min exposure to listed temperature and pressure. The results are presented as mean $\pm$ one standard deviation. On MW and symbols of quantification ions see Table 1.

Component	OLP 1 (494 °C, 6205 kPa)			OLP 2 (578 °C, 7584 kPa)		
	Repeatability for					
	Sample preparation (derivatization)	Intra-day (injection)	Inter-day (over 8 days)	Sample preparation (derivatization)	Intra-day (injection)	Inter-day (over 8 days)
Monocarboxylic acids						
C ₄ MCA	<LOQ ^a	0.09 ± 0.01	<LOQ	0.23 ± 0.01	0.28 ± 0.01	0.23 ± 0.01
C ₅ MCA	<LOQ	0.23 ± 0.01	0.12 ± 0.03	0.27 ± 0.01	0.30 ± 0.01	0.26 ± 0.01
C ₆ MCA	0.34 ± 0.02	0.46 ± 0.01	0.33 ± 0.04	0.48 ± 0.02	0.50 ± 0.01	0.46 ± 0.01
C ₇ MCA	1.42 ± 0.10	1.59 ± 0.01	1.41 ± 0.11	1.30 ± 0.04	1.30 ± 0.01	1.31 ± 0.03
C ₈ MCA	0.45 ± 0.04	0.57 ± 0.01	0.42 ± 0.05	0.53 ± 0.02	0.55 ± 0.01	0.52 ± 0.01
C ₉ MCA	0.28 ± 0.04	0.41 ± 0.01	0.25 ± 0.04	0.42 ± 0.01	0.45 ± 0.01	0.41 ± 0.01
C ₁₀ MCA	0.65 ± 0.05	0.77 ± 0.01	0.63 ± 0.07	0.61 ± 0.02	0.64 ± 0.01	0.60 ± 0.02
C ₁₁ MCA	<LOQ	0.05 ± 0.01	<LOQ	<LOQ	0.08 ± 0.01	0.03 ± 0.01
C ₁₂ MCA	<LOQ	<LOQ	<LOQ	<LOQ	0.03 ± 0.01	<LOQ
C ₁₃ MCA	<LOQ	<LOQ	<LOQ	<LOQ	0.03 ± 0.01	<LOQ
C ₁₄ MCA	<LOQ	<LOQ	<LOQ	<LOQ	0.02 ± 0.01	<LOQ
C ₁₅ MCA	<LOQ	<LOQ	<LOQ	<LOQ	0.02 ± 0.01	<LOQ
C ₁₆ MCA	2.33 ± 0.09	2.54 ± 0.01	2.33 ± 0.08	1.47 ± 0.04	1.43 ± 0.01	1.45 ± 0.02
C ₁₇ MCA	<LOQ	<LOQ	<LOQ	<LOQ	0.02 ± 0.01	<LOQ
C _{18:2} MCA	2.93 ± 0.20	3.21 ± 0.01	3.01 ± 0.19	1.30 ± 0.02	1.27 ± 0.01	1.27 ± 0.03
C _{18:3} MCA	ND ^b	ND	ND	ND	ND	ND
C _{18:1} MCA	2.6 ± 0.2	2.77 ± 0.02	2.5 ± 0.2	1.36 ± 0.04	1.36 ± 0.05	1.34 ± 0.04
C _{18:0} MCA	0.81 ± 0.03	0.93 ± 0.01	0.80 ± 0.04	0.49 ± 0.01	0.52 ± 0.01	0.49 ± 0.01
MCAs total	11.8 ± 0.9	13.62 ± 0.03	11.8 ± 0.9	8.5 ± 0.3	8.80 ± 0.06	8.4 ± 0.2
Glycerol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Monoacylglycerols						
C ₁₆ MAG	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
C _{18:2} MAG	<LOQ	<LOQ	ND	<LOQ	ND	ND
C _{18:1} MAG	<LOQ	<LOQ	ND	ND	ND	ND
C _{18:0} MAG	<LOQ	<LOQ	ND	<LOQ	ND	ND
MAGs total	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Diacylglycerols						
DAG 34 total	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
1,2(2,3)-DAG 36 (S)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
1,2(2,3)-DAG 36 (O)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
1,2(2,3)-DAG 36 (L)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
1,2(2,3)-DAG 36 (Ln)	ND	ND	ND	ND	ND	ND
1,3-DAG 36 (S)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

1,3-DAG 36 (O)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
1,3-DAG 36 (L)	0.46 ± 0.02	0.44 ± 0.01	0.43 ± 0.08	0.10 ± 0.01	0.09 ± 0.01	0.08 ± 0.01
1,3-DAG 36 (Ln)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
DAGs total	0.46 ± 0.02	0.44 ± 0.01	0.43 ± 0.08	0.10 ± 0.01	0.09 ± 0.01	0.08 ± 0.01

Triacylglycerols quantified as [RCO+128]⁺

TAG 50 (P)	<LOQ	<LOQ	<LOQ	0.09 ± 0.01	0.08 ± 0.01	<LOQ
TAG 50 (S)	ND	ND	ND	ND	ND	ND
TAG 50 (O)	<LOQ	<LOQ	ND	ND	ND	ND
TAG 50 (L)	<LOQ	<LOQ	ND	ND	ND	ND
TAG 50 (Ln)	ND	ND	ND	ND	ND	ND
TAG 50 total	<LOQ	<LOQ	<LOQ	0.09 ± 0.01	0.08 ± 0.01	<LOQ
TAG 52 (P)	0.69 ± 0.05	0.70 ± 0.01	0.55 ± 0.03	0.26 ± 0.01	0.21 ± 0.01	0.23 ± 0.01
TAG 52 (S)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
TAG 52 (O)	1.38 ± 0.07	1.31 ± 0.02	1.24 ± 0.06	0.45 ± 0.01	0.41 ± 0.04	0.47 ± 0.08
TAG 52 (L)	3.9 ± 0.8	4.3 ± 0.3	3.55 ± 0.08	1.5 ± 0.3	1.5 ± 0.3	1.2 ± 0.2
TAG 52 (Ln)	<LOQ	<LOQ	<LOQ	0.42 ± 0.01	0.36 ± 0.01	<LOQ
TAG 52 total	6.0 ± 0.8	6 ± 1	5.3 ± 0.2	2.5 ± 0.4	2.4 ± 0.6	1.95 ± 0.05
TAG 54 (O)	2.45 ± 0.08	2.39 ± 0.09	2.1 ± 0.1	0.95 ± 0.06	0.79 ± 0.06	0.81 ± 0.06
TAG 54 (L)	8.4 ± 0.5	8.6 ± 0.2	6.0 ± 0.3	3.3 ± 0.3	3.0 ± 0.5	2.2 ± 0.2
TAG 54 (Ln)	<LOQ	<LOQ	<LOQ	0.67 ± 0.04	0.56 ± 0.07	<LOQ
TAG 54 (S)	<LOQ	<LOQ	<LOQ	0.20 ± 0.01	0.17 ± 0.01	<LOQ
TAG 54 total	10.8 ± 0.5	11 ± 2	8 ± 2	5.1 ± 0.4	4.5 ± 0.6	3.0 ± 0.2
TAGs total	17 ± 2	17 ± 2	9 ± 2	7.7 ± 0.7	6.9 ± 0.7	4.4 ± 0.2

^aBelow the limit of quantification, ^bNot detected.

Suppl. Table 1. Chromatographic methods developed for the simultaneous analysis of MCAs and acylglycerols.

Matrix	Sample preparation	Chromatographic separation	Detection	Injector	Species analyzed	Limitations	Ref.
soybean, palm and coconut oils	HMDS / TFA ^a silylation	GC, DB-5 column, ^b final T = 370 °C	FID, 360 °C	split 1:50, 360 °C	MCAs C ₆ –C ₂₄ , DAGs, TAGs and sterols	emphasis on MCAs only: DAGs and TAGs were not individually identified	[13]
vegetable oils	BSTFA ^c silylation	GC, BPX 5 column, ^b final T = 360 °C	FID, 370 °C	on-column, 45 °C	MCAs C ₁₄ –C ₁₈ , MAGs, DAGs, TAGs, sterols	identification and quantification of species present in standard mixture only, low MW MCAs were not considered	[14]
biodiesel	BSTFA silylation	GC, SAC 5 column, ^b final T = 275 °C	MS, 230 °C ion source, 150 °C quadrupole	splitless, 280 °C	even number MCAs C ₆ –C ₂₄ , glycerol, sterols, MAG C _{18:1}	DAGs or TAGs were not considered	[38]
biodiesel		HPLC	DAD-MS		MCA C ₁₈ , glycerol, MAGs, DAGs and TAGs	unable to measure glycerol, low MW MCAs were not considered	[37]
biodiesel mixtures		non-aqueous RP-LC	UV-Vis		MCAs C _{18:1} , C _{18:2} , C _{18:3} , MAGs, DAGs, TAGs and FAME	low MW MCAs were not considered, quantification was not reported	[40]
biodiesel		UPLC	DAD-ELSD		even number MCAs C ₁₆ –C ₂₀ , glycerol, MAGs, DAGs, TAGs and FAMES	low MW MCAs were not considered, quantification was not reported	[42]
vegetable oils or animal fats		SFC	UV-ELSD		MCAs, FAME, MAGs, DAGs and TAGs	no detailed characterization: separation and quantification as classes of compounds	[41]
biodiesel		SFC	MS-UV-ELSD		glycerol, MCAs C ₁₆ –C ₁₈ and FAME	quantification was not reported, DAGs or TAGs were not considered	[39]

^aHMDS is hexamethyldisilazane, TFA is *trifluoroacetic acid*; ^bAll GC columns listed employed 5 % diphenylmethylpolysiloxane stationary phase; ^cBSTFA is *N,O*-bis(trimethylsilyl) trifluoroacetamide.

Suppl. Table 2. Design matrix for central composite design showing PTV injection experimental parameters.

Run order	Coded levels ^a			Uncoded levels		
	PTV hold time at initial 40 °C, min	Splitless time, min	Oven hold time at initial 35 °C, min	PTV hold time at initial 40 °C, min	Splitless time, min	Oven hold time at initial 35 °C, min
1	1	1	1	2	3.5	3.5
2	-1	-1	1	0.7	1.7	3.5
3	0	0	0	1.35	2.6	2.6
4	-1	-1	-1	0.7	1.7	1.7
5	1	-1	-1	2	1.7	1.7
6	0	0	-1.68	1.35	2.6	1.09
7	-1	1	-1	0.7	3.5	1.7
8	-1.68	0	0	0.26	2.6	2.6
9	0	0	0	1.35	2.6	2.6
10	0	-1.68	0	1.35	1.09	2.6
11	0	0	0	1.35	2.6	2.6
12	0	0	0	1.35	2.6	2.6
13	1	1	-1	2	3.5	1.7
14	0	1.68	0	1.35	4.11	2.6
15	0	0	0	1.35	2.6	2.6
16	1.68	0	0	2.44	2.6	2.6
17	1	-1	1	2	1.7	3.5
18	-1	1	1	0.7	3.5	3.5
19	0	0	0	1.35	2.6	2.6
20	0	0	1.68	1.35	2.6	4.11

^aCodified values: "±1" corresponds to factorial points, "0" corresponds to center points, "±1.68" corresponds to axial points.

Suppl. Table 3. Possible soybean oil acylglycerols formed by various combinations of C_{16:0}, C_{18:0}, C_{18:1}, C_{18:2} and C_{18:3} acyl chains: abbreviations, acyl carbon numbers (ACN), number of double bonds (DB) and MW of expected characteristic ions.

Acyl chains								
Abbreviation		ACN:DB	Systematic name		Trivial name	[RCO] ⁺		
P		16:0	Hexadecanoic		Palmitic	[C ₁₅ H ₃₁ CO] ⁺		
S		18:0	Octadecanoic		Stearic	[C ₁₇ H ₃₅ CO] ⁺		
O		18:1	<i>cis</i> -9-Octadecenoic		Oleic	[C ₁₇ H ₃₃ CO] ⁺		
L		18:2	<i>cis</i> -9,12-Octadecadienoic		Linoleic	[C ₁₇ H ₃₁ CO] ⁺		
Ln		18:3	<i>cis</i> -9,12,15-Octadecatrienoic		Linolenic	[C ₁₇ H ₂₉ CO] ⁺		
Monoacylglycerols								
		ACN:DB	[RCO] ⁺	[RCO+74] ⁺	[RCO+128] ⁺	[M-CH ₃] ⁺		
P		16:0	239	313	367	459		
S		18:0	267	341	395	487		
O		18:1	265	339	393	485		
L		18:2	263	337	391	483		
Ln		18:3	261	335	389	481		
Diacylglycerols								
		ACN:DB	[M-R ₁ COO] ⁺	[M-R ₂ COO] ⁺	[M-R ₁ COO-CH ₂] ⁺	[M-R ₂ COO-CH ₂] ⁺	[M-CH ₃] ⁺	
PP		32:0	385	385	371	371	625	
PS		34:0	385	413	371	399	653	
PO		34:1	385	411	371	397	651	
PL		34:2	385	409	371	395	649	
PLn		34:3	385	407	371	393	647	
SS		36:0	413	413	399	399	681	
SO		36:1	413	411	399	397	679	
SL		36:2	413	409	399	395	677	
OO		36:2	411	411	397	397	677	
SLn		36:3	413	407	399	393	675	
OL		36:3	411	409	397	395	675	
OLn		36:4	411	407	397	393	673	
LL		36:4	409	409	395	395	673	
LLn		36:5	409	407	395	393	671	
LnLn		36:6	407	407	393	393	669	
Triacylglycerols								
		ACN:DB ¹	[R ₁ CO+128] ⁺	[R ₂ CO+128] ⁺	[R ₃ CO+128] ⁺	[M-R ₁ COO] ⁺	[M-R ₂ COO] ⁺	[M-R ₃ COO] ⁺
PPP		48:0	367	367	367	551	551	551
PPS		50:0	367	367	395	579	579	551
POP		50:1	367	393	367	577	551	577
PLP		50:2	367	262	367	575	551	575
PLnP		50:3	367	389	367	573	551	573
PSS		52:0	367	395	395	607	579	579
SOP		52:1	395	393	367	577	579	605
OOP		52:2	393	393	367	577	575	603
SLP		52:2	395	391	367	575	579	603
LOP		52:3	391	393	367	577	575	601

S _{Ln} P	52:3	395	389	367	573	579	601
LLP	52:4	391	391	367	575	575	599
O _{Ln} P	52:4	393	389	367	573	577	599
L _n LP	52:5	389	391	367	575	573	597
L _n L _n P	52:6	389	389	367	573	573	595
SSS	54:0	395	395	395	607	607	607
SOS	54:1	395	393	395	605	607	605
SLS	54:2	395	391	395	603	607	603
SOO	54:2	395	393	393	603	605	605
OOO	54:3	393	393	393	603	603	603
SLO	54:3	395	391	393	601	605	603
SSL _n	54:3	395	395	389	601	601	607
OLO	54:4	393	391	393	601	603	601
SLL	54:4	395	391	391	599	603	603
SOL _n	54:4	395	393	389	599	601	605
OLL	54:5	393	391	391	599	601	601
O _{Ln} O	54:5	393	389	393	599	603	599
SLL _n	54:5	395	391	389	597	601	603
LLL	54:6	391	391	391	599	599	599
S _{Ln} L _n	54:6	395	389	389	595	601	601
OLL _n	54:6	393	391	389	597	599	601
LLL _n	54:7	391	391	389	597	597	599
O _{Ln} L _n	54:7	393	389	389	595	599	599
LL _n L _n	54:8	391	389	389	595	597	597
L _n L _n L _n	54:9	389	389	389	595	595	595

Suppl. Table 4. Calibration parameters for MCAs upon derivatization with MSTFA determined by HTGC-FID/MS in SITI. The MS quantitation was performed using ions listed in Table 1.

Compound	FID				TIC (SITI)				TIC/EIC (SITI)				SIM/EIC (SITI)			
	slope	intercept	S _y ^a	R ²	slope	intercept	S _y	R ²	slope	intercept	S _y	R ²	slope	intercept	S _y	R ²
Butanoic acid	0.0080	0.4651	0.1148	0.9756	0.0097	0.9342	0.1971	0.9517	0.0100	0.0383	0.0469	0.9978	0.0102	0.0359	0.0496	0.9976
Pentanoic acid	0.0163	0.0009	0.0146	0.9998	0.0234	0.0504	0.0538	0.9990	0.0098	0.0208	0.0350	0.9979	0.0099	0.0201	0.0350	0.9980
Hexanoic acid	0.0166	0.0026	0.0132	0.9999	0.0247	0.0721	0.0784	0.9980	0.0094	0.0252	0.0318	0.9981	0.0096	0.0248	0.0332	0.9980
Heptanoic acid	0.0174	0.0245	0.0185	0.9998	0.0264	0.0931	0.0779	0.9984	0.0097	0.0277	0.0410	0.9973	0.0098	0.0311	0.0383	0.9977
Octanoic acid	0.0178	0.0337	0.0219	0.9997	0.0276	0.0920	0.0779	0.9984	0.0100	0.0228	0.0359	0.9979	0.0101	0.0263	0.0381	0.9977
Nonanoic acid	0.0158	0.0319	0.0212	0.9997	0.0248	0.0937	0.0713	0.9984	0.0089	0.0232	0.0395	0.9969	0.0088	0.0278	0.0319	0.9980
Decanoic acid	0.0171	0.0368	0.0210	0.9997	0.0279	0.0956	0.0790	0.9983	0.0098	0.0207	0.0402	0.9971	0.0095	0.0267	0.0414	0.9967
Undecanoic acid	0.0204	0.0401	0.0245	0.9997	0.0338	0.1010	0.0987	0.9982	0.0116	0.0198	0.0285	0.9990	0.0111	0.0282	0.0450	0.9972
Dodecanoic acid	0.0209	0.0433	0.0244	0.9997	0.0349	0.1112	0.0997	0.9985	0.0118	0.0179	0.0567	0.9964	0.0111	0.0291	0.0360	0.9984
Tridecanoic acid	0.0211	0.0445	0.0254	0.9997	0.0359	0.1055	0.1130	0.9982	0.0119	0.0158	0.0262	0.9993	0.0115	0.0186	0.0261	0.9992
Tetradecanoic acid	0.0214	0.0462	0.0266	0.9997	0.0367	0.1129	0.1122	0.9984	0.0121	0.0119	0.0478	0.9977	0.0106	0.0303	0.0460	0.9974
Pentadecanoic acid	0.0213	0.0403	0.0234	0.9997	0.0374	0.0800	0.0992	0.9984	0.0119	0.0052	0.0400	0.9979	0.0102	0.0229	0.0377	0.9974
Hexadecanoic acid	0.0225	0.0447	0.0244	0.9997	0.0397	0.1067	0.1225	0.9979	0.0125	0.0101	0.0329	0.9987	0.0103	0.0290	0.0426	0.9968
Heptadecanoic acid	0.0217	0.0430	0.0241	0.9998	0.0387	0.0747	0.1013	0.9986	0.0118	0.0002	0.0400	0.9981	0.0091	0.0236	0.0438	0.9962
Octadecanoic acid	0.0219	0.0543	0.0312	0.9997	0.0384	0.1543	0.1371	0.9984	0.0117	0.0090	0.0188	0.9997	0.0085	0.0452	0.0696	0.9930
Oleic acid	0.0161	0.0161	0.0622	0.9965	0.0244	0.1975	0.2152	0.9821	0.0055	-0.0007	0.0225	0.9968	0.0044	0.0076	0.0158	0.9975
Linoleic acid	0.0267	-0.1238	0.0965	0.9970	0.0392	-0.1739	0.3704	0.9800	0.0024	-0.0025	0.0053	0.9991	0.0020	0.0010	0.0084	0.9968
Linolenic acid	0.0099	0.1339	0.1030	0.9766	0.0179	0.1564	0.3614	0.9172	0.0008	-0.0003	0.0023	0.9983	0.0007	0.0009	0.0026	0.9970

^aThe standard error for the y estimate.

Suppl. Table 5. Calibration parameters for acylglycerols upon derivatization with MSTFA determined by HTGC-FID/MS. The MS quantitation was performed using ions listed in Table 1.

Compound	FID				TIC (SITI)				TIC/EIC (SITI)				SIM/EIC (SITI)			
	slope	intercept	S _y ^a	R ²	slope	intercept	S _y	R ²	slope	intercept	S _y	R ²	slope	intercept	S _y	R ²
Glycerol	0.0143	0.0002	0.0025	0.9997	0.0410	0.0045	0.0139	0.9989	0.0108	0.0011	0.0043	0.9987	0.0114	0.0007	0.0040	0.9990
Monopalmitin	0.0208	0.0122	0.0110	0.9999	0.0298	-0.0552	0.0341	0.9995	0.1178	-0.3534	0.2005	0.9991	0.0800	-0.0220	0.1630	0.9987
Monostearin	0.0204	0.0118	0.0103	0.9999	0.0309	-0.0406	0.0577	0.9987	0.1099	-0.2426	0.3192	0.9974	0.0632	0.1220	0.2452	0.9954
Monoolein	0.0205	0.0547	0.1563	0.9785	0.0141	-0.0211	0.0623	0.9560	0.0417	-0.1030	0.0935	0.9984	0.0273	0.0071	0.0845	0.9970
Monolinolein	0.0221	0.0757	0.0221	0.9984	0.0100	0.0967	0.0744	0.9837	0.0130	-0.0437	0.0300	0.9984	0.0090	-0.0077	0.0235	0.9980
Dipalmitin	0.0199	0.0167	0.0030	0.9999	0.0321	-0.1084	0.0463	0.9980	0.0610	-0.2006	0.1049	0.9977	0.0443	-0.0983	0.0641	0.9984
Distearin	0.0184	0.0123	0.0049	0.9999	0.0353	-0.0858	0.0575	0.9976	0.0802	-0.1963	0.0832	0.9992	0.0509	-0.0443	0.1037	0.9969
Diolen	0.0195	0.0271	0.0179	0.9998	0.0180	-0.1345	0.0555	0.9978	0.0203	-0.0765	0.0416	0.9971	0.0122	-0.0192	0.0154	0.9989
Dilinolein	0.0200	0.0245	0.0180	0.9998	0.0050	-0.0721	0.0625	0.9967	0.0071	-0.0325	0.0216	0.9925	0.0089	-0.0370	0.0205	0.9956
Quantified as [RCO+128] ⁺																
Tripalmitin	0.0161	0.0129	0.0062	0.9998	0.0157	-0.0481	0.0283	0.9972	0.0133	-0.0278	0.0370	0.9983	0.0107	-0.0178	0.0246	0.9960
Tristearin	0.0125	0.0085	0.0069	0.9995	0.0101	-0.0309	0.0214	0.9912	0.0116	-0.0373	0.0464	0.9965	0.0077	-0.0147	0.0167	0.9961
Triolein	0.0119	0.0072	0.0060	0.9997	0.0044	-0.0048	0.0150	0.9849	0.0049	-0.0516	0.0240	0.9970	0.0037	-0.0290	0.0181	0.9871
Trilinolein	0.0081	0.0078	0.0149	0.9995	0.0012	-0.0109	0.0192	0.9663	0.0008	-0.0111	0.0106	0.9842	0.0005	-0.0037	0.0018	0.9895
Trilinolenin	0.0059	0.1507	0.3684	0.9920	0.0008	-0.0856	0.0425	0.9951	0.0008	-0.0095	0.0351	0.9351	0.0004	-0.0042	0.0014	0.9922
Triarachidin	0.0080	-0.0004	0.0095	0.9989	0.0053	-0.0113	0.0152	0.9722	0.0044	-0.0534	0.0332	0.9861	0.0025	-0.0212	0.0155	0.9786
Quantified as [M-RCOO] ⁺																
Tripalmitin	NA ^b	NA	NA	NA	NA	NA	NA	NA	0.0254	-0.1200	0.0524	0.9933	0.0213	-0.0213	0.0510	0.9948
Tristearin	NA	NA	NA	NA	NA	NA	NA	NA	0.0168	-0.0862	0.0193	0.9883	0.0142	-0.0663	0.0416	0.9913
Triolein	NA	NA	NA	NA	NA	NA	NA	NA	0.0136	-0.0955	0.0159	0.9824	0.0116	-0.0780	0.0590	0.9827
Trilinolein	NA	NA	NA	NA	NA	NA	NA	NA	0.0033	-0.0290	0.0104	0.9884	0.0027	-0.0155	0.0118	0.9818
Trilinolenin	NA	NA	NA	NA	NA	NA	NA	NA	0.0015	-0.0446	0.0100	0.9967	0.0009	-0.0078	0.0052	0.9853
Triarachidin	NA	NA	NA	NA	NA	NA	NA	NA	0.0015	-0.0016	0.0019	0.8682	0.0037	-0.0183	0.0222	0.9749

^aThe standard error for the y estimate, ^bNot applicable as those values provided above in the table.

Suppl. Table 6. Composition of OLPs quantified by TIC/EIC and FID. The OLP samples were obtained after 5 min exposure to listed temperature and pressure. The results are presented as mean of weight % $\pm$ one standard deviation of three sample preparations (derivatizations). On MW and abbreviation of quantification ions see Table 1. Composition of OLP 1 and 2 is reported in Tables 4 and 5.

Component	Composition weight % of OLP							
	Quantified by TIC/EIC				Quantified by FID			
	OLP 3	OLP 4	OLP 5	OLP 6	OLP 3	OLP 4	OLP 5	OLP 6
	470 °C 2930 kPa	486 °C 5516 kPa	590 °C 9997 kPa	620 °C 4826 kPa	470 °C 2930 kPa	486 °C 5516 kPa	590 °C 9997 kPa	620 °C 4826 kPa
Monocarboxylic acids								
Butanoic acid	<LOQ ^a	0.17 $\pm$ 0.01	0.12 $\pm$ 0.01	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Pentanoic acid	0.09 $\pm$ 0.01	0.23 $\pm$ 0.01	0.15 $\pm$ 0.01	<LOQ	0.16 $\pm$ 0.01	0.29 $\pm$ 0.01	0.17 $\pm$ 0.01	0.21 $\pm$ 0.02
Hexanoic acid	0.20 $\pm$ 0.01	0.41 $\pm$ 0.01	0.28 $\pm$ 0.01	<LOQ	0.20 $\pm$ 0.01	0.39 $\pm$ 0.01	0.28 $\pm$ 0.04	<LOQ
Heptanoic acid	0.72 $\pm$ 0.01	1.00 $\pm$ 0.03	0.97 $\pm$ 0.04	0.47 $\pm$ 0.02	0.69 $\pm$ 0.01	0.96 $\pm$ 0.02	0.93 $\pm$ 0.04	0.51 $\pm$ 0.01
Octanoic acid	0.25 $\pm$ 0.01	0.45 $\pm$ 0.01	0.35 $\pm$ 0.02	<LOQ	0.27 $\pm$ 0.01	0.49 $\pm$ 0.01	0.37 $\pm$ 0.02	0.26 $\pm$ 0.01
Nonanoic acid	0.20 $\pm$ 0.01	0.37 $\pm$ 0.01	0.25 $\pm$ 0.01	<LOQ	0.20 $\pm$ 0.01	0.39 $\pm$ 0.01	0.24 $\pm$ 0.01	0.21 $\pm$ 0.01
Decanoic acid	0.33 $\pm$ 0.01	0.48 $\pm$ 0.01	0.46 $\pm$ 0.02	<LOQ	0.34 $\pm$ 0.01	0.50 $\pm$ 0.01	0.48 $\pm$ 0.02	0.25 $\pm$ 0.01
Undecanoic acid	<LOQ	0.05 $\pm$ 0.01	<LOQ	<LOQ	0.07 $\pm$ 0.01	0.14 $\pm$ 0.01	0.09 $\pm$ 0.01	<LOQ
Dodecanoic acid	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.03 $\pm$ 0.01	<LOQ	<LOQ
Tridecanoic acid	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Tetradecanoic acid	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Pentadecanoic acid	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Hexadecanoic acid	0.95 $\pm$ 0.02	1.36 $\pm$ 0.07	1.17 $\pm$ 0.06	1.51 $\pm$ 0.03	0.95 $\pm$ 0.02	1.32 $\pm$ 0.08	1.18 $\pm$ 0.05	1.53 $\pm$ 0.01
Heptadecanoic acid	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Linoleic acid	1.13 $\pm$ 0.01	1.49 $\pm$ 0.04	1.26 $\pm$ 0.06	1.43 $\pm$ 0.02	0.56 $\pm$ 0.02	0.55 $\pm$ 0.01	0.55 $\pm$ 0.02	0.84 $\pm$ 0.02
Linolenic acid	ND ^b	ND	ND	ND	ND	ND	ND	ND
Oleic acid	0.97 $\pm$ 0.03	1.41 $\pm$ 0.03	1.23 $\pm$ 0.03	1.73 $\pm$ 0.01	1.63 $\pm$ 0.05	2.56 $\pm$ 0.07	2.09 $\pm$ 0.10	2.56 $\pm$ 0.04
Octadecanoic acid	0.30 $\pm$ 0.01	0.46 $\pm$ 0.03	0.39 $\pm$ 0.02	0.51 $\pm$ 0.02	0.29 $\pm$ 0.01	0.47 $\pm$ 0.04	0.40 $\pm$ 0.02	0.50 $\pm$ 0.03
MCAs total	5.15 $\pm$ 0.07	7.8 $\pm$ 0.3	6.6 $\pm$ 0.3	5.6 $\pm$ 0.4	5.4 $\pm$ 0.2	8.19 $\pm$ 0.3	6.87 $\pm$ 0.3	6.86 $\pm$ 0.05
Glycerol	<LOQ	<LOQ	<LOQ	ND	ND	ND	ND	ND
Monoacylglycerols								
Monopalmitin	<LOQ	<LOQ	<LOQ	ND	<LOQ	<LOQ	<LOQ	<LOQ
Monolinolein	ND	<LOQ	<LOQ	ND	<LOQ	0.05 $\pm$ 0.01	0.09 $\pm$ 0.01	0.23 $\pm$ 0.04
Monoolein	ND	ND	ND	ND	<LOQ	<LOQ	<LOQ	<LOQ
Monostearin	<LOQ	<LOQ	<LOQ	ND	<LOQ	<LOQ	<LOQ	<LOQ
MAGs total	<LOQ	<LOQ	<LOQ	ND	<LOQ	0.05 $\pm$ 0.01	0.09 $\pm$ 0.01	0.23 $\pm$ 0.04
Diacylglycerols								
DAG, ACN 34 total	<LOQ	0.19 $\pm$ 0.01	<LOQ	ND	0.05 $\pm$ 0.01	0.06 $\pm$ 0.01	0.04 $\pm$ 0.01	<LOQ
1,2(2,3)-DAG, ACN ^c 36								
C _{18:0} acyl chain	<LOQ	<LOQ	<LOQ	ND	NA ^d	NA	NA	NA
C _{18:1} acyl chain	<LOQ	0.04 $\pm$ 0.01	<LOQ	ND	NA	NA	NA	NA
C _{18:2} acyl chain	0.12 $\pm$ 0.01	0.09 $\pm$ 0.01	0.08 $\pm$ 0.01	ND	NA	NA	NA	NA
C _{18:3} acyl chain	<LOQ	0.04 $\pm$ 0.01	<LOQ	ND	NA	NA	NA	NA
1,2(2,3)-DAG, ACN 36 total	0.12 $\pm$ 0.01	0.17 $\pm$ 0.01	0.08 $\pm$ 0.01	ND	0.25 $\pm$ 0.01	0.15 $\pm$ 0.01	0.12 $\pm$ 0.01	<LOQ
1,3-DAG, ACN 36								
C _{18:0} acyl chain	<LOQ	<LOQ	<LOQ	ND	NA	NA	NA	NA
C _{18:1} acyl chain	0.06 $\pm$ 0.01	0.05 $\pm$ 0.01	<LOQ	ND	NA	NA	NA	NA

C _{18:2} acyl chain	0.13 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	ND	NA	NA	NA	NA
C _{18:3} acyl chain	0.08 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	ND	NA	NA	NA	NA
1,3-DAG, ACN 36 total	0.27 ± 0.02	0.22 ± 0.02	0.18 ± 0.01	ND	0.22 ± 0.01	0.26 ± 0.01	0.18 ± 0.01	<LOQ
DAGs total	0.39 ± 0.01	0.58 ± 0.02	0.26 ± 0.02	ND	0.52 ± 0.01	0.47 ± 0.01	0.34 ± 0.01	<LOQ
Triacylglycerols								
Quantified as [RCO+128]⁺								
TAG, ACN 50								
C _{16:0} acyl chain	0.28 ± 0.04	0.14 ± 0.01	0.18 ± 0.01	ND	NA	NA	NA	NA
C _{18:0} acyl chain	ND	ND	ND	ND	NA	NA	NA	NA
C _{18:1} acyl chain	<LOQ	<LOQ	<LOQ	ND	NA	NA	NA	NA
C _{18:2} acyl chain	0.89 ± 0.3	0.43 ± 0.02	0.69 ± 0.05	ND	NA	NA	NA	NA
C _{18:3} acyl chain	ND	ND	ND	ND	NA	NA	NA	NA
TAG, ACN 50 total	1.1 ± 0.3	0.57 ± 0.02	0.9 ± 0.4	ND	0.73 ± 0.08	0.33 ± 0.01	0.41 ± 0.02	<LOQ
TAG, ACN 52								
C _{16:0} acyl chain	0.92 ± 0.06	0.50 ± 0.02	0.52 ± 0.05	ND	NA	NA	NA	NA
C _{18:0} acyl chain	<LOQ	<LOQ	<LOQ	ND	NA	NA	NA	NA
C _{18:1} acyl chain	1.6 ± 0.2	0.90 ± 0.07	0.93 ± 0.05	ND	NA	NA	NA	NA
C _{18:2} acyl chain	5 ± 0.64	2.9 ± 0.38	2.74 ± 0.10	ND	NA	NA	NA	NA
C _{18:3} acyl chain	1.2 ± 0.2	0.76 ± 0.04	0.70 ± 0.01	ND	NA	NA	NA	NA
TAG, ACN 52 total	8.7 ± 0.79	5.1 ± 0.3	4.9 ± 0.52	ND	11.6 ± 0.3	6.07 ± 0.08	5.9 ± 0.3	0.52 ± 0.02
TAG, ACN 54								
C _{18:0} acyl chain	0.59 ± 0.07	0.33 ± 0.03	0.38 ± 0.02	ND	NA	NA	NA	NA
C _{18:1} acyl chain	3.2 ± 0.3	1.78 ± 0.07	1.81 ± 0.08	ND	NA	NA	NA	NA
C _{18:2} acyl chain	11 ± 2	6.4 ± 0.56	5.9 ± 0.4	ND	NA	NA	NA	NA
C _{18:3} acyl chain	2.3 ± 0.2	1.39 ± 0.09	1.24 ± 0.08	ND	NA	NA	NA	NA
TAG, ACN 54 total	17 ± 2	10 ± 0.6	9.3 ± 0.38	ND	23.4 ± 0.5	12.2 ± 0.6	11.9 ± 0.2	1.06 ± 0.04
TAGs total	27 ± 3	15.49 ± 0.83	15.0 ± 0.5	ND	35.7 ± 0.8	18.6 ± 0.7	18.2 ± 0.5	1.58 ± 0.05

^aBelow the limit of quantification, ^bNot detected, ^cAcyl carbon number, ^dNot applicable for FID quantification.

Suppl. Table 7. Triacylglycerol composition in OLPs quantified by TIC/EIC using [M-RCOO]⁺. The OLP samples were obtained after 5 min exposure to listed temperature and pressure. The results are presented as mean weight % $\pm$ one standard deviation of three sample preparations (derivatizations).

Component	Quantification ion	Calibration ion	Composition wt. % of OLP					
			Quantified by TIC/EIC using [M-RCOO] ⁺					
			OLP 1 494 °C 6205 kPa	OLP 2 578 °C 7584 kPa	OLP 3 470 °C 2930 kPa	OLP 4 486 °C 5516 kPa	OLP 5 590 °C 9997 kPa	OLP 6 620 °C 4826 kPa
Triacylglycerols, ACN ^a								
TAG, ACN 50								
combination of two C _{16:0} acyl chains	551	551	<LOQ ^b	0.11 ± 0.01	0.36 ± 0.03	0.20 ± 0.01	0.23 ± 0.01	ND ^c
combination of C _{16:0} and C _{18:0} acyl chains	579	551	ND	ND	ND	ND	ND	ND
combination of C _{16:0} and C _{18:1} acyl chains	577	551	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	ND
combination of C _{16:0} and C _{18:2} acyl chains	575	551	<LOQ	<LOQ	0.20 ± 0.03	0.11 ± 0.01	<LOQ	ND
combination of C _{16:0} and C _{18:3} acyl chains	573	551	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	ND
TAG, ACN 50 total			<LOQ	0.11 ± 0.01	0.56 ± 0.05	0.31 ± 0.01	0.23 ± 0.01	ND
TAG, ACN 52								
combination of C _{16:0} and C _{18:0} acyl chains	579	551	<LOQ	0.15 ± 0.01	0.49 ± 0.03	0.27 ± 0.01	0.31 ± 0.02	ND
combination of C _{16:0} and C _{18:1} acyl chains	577	551	0.87 ± 0.06	0.47 ± 0.06	1.8 ± 0.2	0.96 ± 0.09	0.99 ± 0.05	<LOQ
combination of C _{16:0} and C _{18:2} acyl chains	575	551	0.71 ± 0.03	0.37 ± 0.09	1.4 ± 0.1	0.76 ± 0.01	0.73 ± 0.04	<LOQ
combination of C _{16:0} and C _{18:3} acyl chains	573	551	<LOQ	0.11 ± 0.06	0.35 ± 0.03	0.20 ± 0.01	0.21 ± 0.01	ND
combination of two C _{18:1} or C _{18:0} and C _{18:2} acyl chains	603	603	<LOQ	0.13 ± 0.01	0.35 ± 0.03	0.21 ± 0.01	0.26 ± 0.01	ND
combination of two C _{18:2} or C _{18:1} and C _{18:3} acyl chains	599	599	1.04 ± 0.02	0.48 ± 0.04	1.8 ± 0.2	0.94 ± 0.03	0.95 ± 0.05	ND
combination of two C _{18:3} acyl chains	595	595	ND	ND	<LOQ	<LOQ	<LOQ	ND
combination of C _{18:1} and C _{18:2} or C _{18:0} and C _{18:3} acyl chains	601	603	<LOQ	0.16 ± 0.01	0.50 ± 0.03	0.27 ± 0.01	0.33 ± 0.01	ND
combination of C _{18:1} and C _{18:2} acyl chains	597	599	<LOQ	0.19 ± 0.01	0.64 ± 0.05	0.37 ± 0.02	0.39 ± 0.01	ND
combination of C _{18:0} and C _{18:1} acyl chains	605	607	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	ND
combination of two C _{18:0} acyl chains	607	607	ND	ND	<LOQ	ND	<LOQ	ND
TAG, ACN 52 total			2.6 ± 0.7	2.1 ± 0.3	7.3 ± 0.6	3.98 ± 0.07	4.2 ± 0.2	ND
TAG, ACN 54								
combination of two C _{18:1} or C _{18:0} and C _{18:2} acyl chains	603	603	1.9 ± 0.2	1.03 ± 0.04	3.9 ± 0.4	2.22 ± 0.02	2.2 ± 0.2	<LOQ
combination of two C _{18:2} or C _{18:1} and C _{18:3} acyl chains	599	599	5.3± 0.7	3.0 ± 0.2	11.9 ± 0.8	6.7 ± 0.3	5.7 ± 0.4	<LOQ
combination of two C _{18:3} acyl chains	595	595	<LOQ	<LOQ	2.05 ± 0.13	1.20 ± 0.03	<LOQ	ND
combination of C _{18:1} and C _{18:2} or C _{18:0} and C _{18:3} acyl chains	601	603	1.9 ± 0.2	1.00 ± 0.03	3.9 ± 0.3	2.17 ± 0.02	2.0 ± 0.2	<LOQ
combination of C _{18:1} and C _{18:2} acyl chains	597	599	1.9 ± 0.34	1.02 ± 0.03	3.98 ± 0.4	2.16 ± 0.01	1.82 ± 0.08	ND
combination of C _{18:0} and C _{18:1} acyl chains	605	607	0.8 ± 0.2	0.38 ± 0.01	1.43 ± 0.08	0.78 ± 0.03	0.82 ± 0.08	<LOQ
combination of two C _{18:0} acyl chains	607	607	<LOQ	0.11 ± 0.01	0.32 ± 0.05	0.20 ± 0.01	0.21 ± 0.01	ND
TAG, ACN 54 total			12 ± 2	6.5 ± 0.2	27 ± 2	15.4 ± 0.3	12.7 ± 0.8	<LOQ
TAGs total			15 ± 2	8.7 ± 0.3	35 ± 3	19.7 ± 0.5	17 ± 1	<LOQ

^aAcyl carbon number, ^bBelow the limit of quantification, ^cNot detected.