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Method development for the characterization of biofuel intermediate products using gas chromatography with simultaneous mass spectrometric and flame ionization detections

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ABSTRACT

Accurate analytical methods are required to develop and evaluate the quality of new renewable transportation fuels and intermediate organic liquid products (OLPs). Unfortunately, existing methods developed for the detailed characterization of petroleum products, are not accurate for many of the OLPs generated from non-petroleum feedstocks. In this study, a method was developed and applied to the detailed characterization of complex OLPs formed during triacylglyceride (TG) pyrolysis which is the basis for generating one class of emerging biofuels. This method uses gas chromatography coupled simultaneously with flame ionization and mass spectrometry detectors (GC–FID/MS). The FID provided accurate quantification of carbonaceous species while MS enabled identification of unknown compounds. A programed temperature vaporizer using a 25 °C, 0.1 min, 720 °C min^{-1}, 350 °C, 5 min temperature program is employed which minimizes compound discrimination better than the more commonly utilized split/splitless injector, as verified with injections at 250 and 350 °C. Two standard mixtures featuring over 150 components are used for accurate identification and a designed calibration standard accounts for compound discrimination at the injector and differing FID responses of various classes of compounds. This new method was used to identify and quantify over 250 species in OLPs generated from canola oil, soybean oil, and canola methyl ester (CME). In addition to hydrocarbons, the method was used to quantify polar (upon derivatization) and unidentified species, plus the unresolved complex mixture that has not typically been determined in previous studies. Repeatability of the analytical method was below 5% RSD for all individual components. Using this method, the mass balance was closed for samples derived from canola and soybean oil but only ca. 77 wt% of the OLP generated from CME could be characterized. The ability to close the mass balance depended on sample origin, demonstrating the need for an accurate quantification method for biofuels at various stages of production.

1. Introduction

A variety of biofuels with different chemical compositions are being developed from triacylglycerides (TGs) using assorted processes [1–12]. One strategy is to derive biofuels from the organic liquid product (OLP) generated during TG pyrolysis [2,4,7,13]. These fuels are more compatible with their existing petroleum-based diesel and kerosene fuel analogs than biodiesel [2,4,7,13] and thus show significant future potential. To assist in process development, scale-up, and commercialization, accurate identification and quantification of OLP mixtures as well as verification of the mass balance are critical. If the analysis is incomplete, key components that could be detrimental to fuel quality (such as high-MW chemicals formed via oligomerization during pyrolysis) can be missed.

The only ASTM detailed analytical procedure developed for transportation fuels, D6730, was developed strictly for petroleum based fuel samples [14]. Unfortunately, this method is not directly applicable for characterization of organic mixtures of relatively unknown compositions and may not provide complete, accurate quantification of the mixture's composition.

Various studies that have evaluated OLPs generated by the pyrolysis of TG oils have reported the identification and quantification of selected species [2–9]. However, as discussed below, the approaches used to qualitatively and quantitatively determine the individual organic compounds in OLPs have numerous limitations [2–6,8,9,15–21]. Thus, a direct comparison between the reported results and even an assessment of sample composition is difficult.

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Typical analytical methods have included NMR [15,17,20], FTIR [3,6,15,16,21], and/or chromatographic techniques [5,6,8,17,21]. The advantage of NMR and FTIR is the overall characterization of the OLP. But these methods can only provide an estimate of the total content of classes of compounds (e.g., carbonyl compounds).

GC–MS or GC with flame ionization detector (GC–FID) are the most common methods employed for characterization of biofuels and petroleum products [2–9,14]. The problem affecting analyte identification is the complexity of the samples generated from sources other than petroleum. Identification using FID is performed on the basis of the analyte's retention time or retention index matched to that of a corresponding standard or by comparison to a GC–MS analysis performed on another instrument [4,8,18]. Matching the retention times may be a good first-order approximation, but it becomes inconclusive for the identification of hundreds of species. The matching of GC–FID to GC–MS complex chromatograms obtained for the same samples on different instruments may also be inaccurate due to differences in instrumental conditions.

The quantification in previous studies characterizing OLPs typically targeted only selected species, employed normalization (assuming all species as GC-elutable, i.e., 100%), and often disregarded the unresolved complex mixture (UCM) [14,16,22,23]. For characterization of a large number of analytes, the standard approach is to use the FID relative response factors (RFs) of selected species (based on the assumption that the FID response is directly proportional to the number of carbon atoms introduced [24]). However, the FID response in GC analysis may be negatively affected by the discrimination at the injector as well as by the presence of heteroatoms in the analyte [24]. Therefore, it is necessary to address these limitations when selecting appropriate standards. Unfortunately, a number of prior OLP studies did not report quantification conditions (e.g., whether standards were employed or how the RFs were obtained) [2,4–6,18,19], thus, it is not possible to ascertain if these limitations were considered.

As mentioned above, a commonly used quantification approach is to normalize the peak areas of MS or FID chromatograms [14,16,22]. This approach is correct for petroleum fuels because the composition is well known and virtually all of the species have been shown to be GC elutable (e.g., ASTM method D6730 designed for spark ignition engine fuels) [14]. This may not be true for OLPs generated during TG pyrolysis. The problem of area normalization is even more pronounced when quantification is based on MS without or only with a limited number of quantification standards since analyte ionization leads to varied RFs of different components. Unless a calibration is performed for all targeted species, the correct quantification may not be achieved [2,16,17,22].

Another source of quantification errors may be discrimination at the injection port [14,25]. ASTM D6730 employs split injection at 250 °C and requires the establishment of split injection linearity. When using hot split injection discrimination will occur and has to be corrected by applying response factors. One solution is to use an on-column injection to eliminate injection discrimination. However, this type of injection is more applicable for samples with low analyte concentration which easily volatilize and will not cause a buildup of non-volatile materials on the column. This technique is not suitable for highly concentrated fuel or biofuel samples that may containing heavier components. Another solution is to use programed temperature vaporizer (PTV) injection to minimize injection discrimination [25]. However, only a few studies have employed PTV for injection of small sample volumes and those studies were not targeting fuels [25]. The quantification of OLPs may also be incomplete if the UCM is not estimated. Unresolved branched alkanes, cycloalkanes, aromatics, and alkenes are common constituents of the UCM and are expressed by a baseline "hump" in the GC analysis of complex matrices generated from biomass [26–28]. While resolution and identification of individual compounds in the UCM may not be possible, estimating the overall concentration of the UCM is necessary for complete mass balance closure.

Two-dimensional GC (GC × GC) has been popular for the analysis of complex materials [29–32]. The increased peak capacity and speed in GC × GC resulting from its orthogonal design can solve some inaccurate identification issues. However a true orthogonal system that allows for the separation of compounds with similar properties is difficult to achieve since the separation mechanism of commercially available GC columns is not truly orthogonal. As with 1D-GC, the increased peak capacity and thus the separation power is achieved while sacrificing analysis time [33]. Moreover, the simultaneous set up of detectors suitable to identify and/or quantify carbonaceous species is not commercially available. Therefore in-house system modifications or complicated switching mechanisms between detectors must be employed to accurately identify (using MS) and quantify (using FID) the large number of components present in complex matrices.

As mentioned above while there are numerous studies documenting the composition of biofuels and OLPs using various analytical methods [10,34,35], the majority of these studies employed methods that are either derived from petroleum products (not applicable to biofuels without further validation), or are not fully quantitative (employ only area normalization for quantification). Thus these results may not be fully accurate. To our knowledge no comprehensive method addressing detailed characterization of biofuels and their OLPs has been previously reported.

The aim of our work was to develop an accurate method for detailed identification and quantification of OLPs generated by TG pyrolysis using GC–FID/MS. The accuracy of compound identification was improved by the parallel setup of both detectors within one instrument. This method minimized errors caused by retention time shifts in compound identification and accounted for the UCM and non GC-elutable fractions that may be present in the sample. The injection conditions using split/splitless (SS) and programed temperature vaporizer (PTV) injections, and a column temperature program were optimized to minimize discrimination and improve separation. Accurate quantification was thus ensured. The developed method was applied to OLPs generated by the pyrolysis of canola oil, soybean oil, and canola methyl esters (CME).

2. Experimental

2.1. Materials

Three feedstocks were used to generate OLPs. Soybean oil was primarily obtained from the Northwood Mills, ND oil seed crushing facility. Superdegummed canola oil and CME were obtained from the feed and product lines of a canola oil biodiesel facility located in Velva, ND. The OLPs were generated by the pyrolysis of each oil under conditions that produced a mixture conductive to further processing, e.g., distillation, decarboxylation, etc., into jet and diesel transportation fuels. Details on the experimental conditions used to generate these samples were reported previously [7,13]. All samples were stored at 4 °C until analysis.

GC grade methylene chloride was purchased from Fisher (Waltham, MA, USA). Two mixtures, designated herein as Mixes 1 and 2, were formulated to facilitate the detailed identification of chemical species in the OLP. The compounds present in Mixes 1 and 2, their retention with relative retention times to the internal standard of benzene-d_6 (I.S.1), and representative MS ions are listed in the supplementary material (Tables S1 and S2, chromatograms are shown in Figs. S1 and S2). Mix 1 consisted of a homologous series of *n*-alkanes, aromatics, and Alphagaz PIANO mixtures (in 1:1:1:1:1 ratios) of paraffins, isoparaffins, aromatics, naphthenes, and olefins

(Supelco, Bellefonte, PA, USA). Mix 2 was composed of homologous series of alkenes and fatty acid methyl esters (FAMEs), qualitative reformate, alkylate, and naphtha standards. Food industry fatty acid methyl esters (FAMEs) mix and NLEA FAME mix were purchased from Restek (Bellefonte, PA, USA). Naphtha, reformate, and alkylate qualitative reference standards; and crude oil qualitative and quantitative standards (ASTM D5134) were obtained from Supelco.

For quantification of the OLPs, a mixture was prepared from analytical grade standards consisting of a full series of *n*-alkanes ($C_{5–18}$), selected alkenes ($C_{6, 9, 14, 18}$), FAMEs ($C_{3, 6, 10, 14, 18}$), and aromatics (benzene, toluene, *p*-xylene, 1,2,4-trimethylbenzene, indane, and naphthalene). *n*-Pentane and benzene were purchased from OmniSolv (Gibbstwon, NJ, USA). *n*-Heptane, *n*-decane, *n*-dodecane, and methyl propionate were purchased from Acros (Morris Plains, NJ, USA). *n*-Undecane and *n*-pentadecane were obtained from TCI America (Portland, OR, USA). *n*-Nonene, naphthalene, ethylbenzene, *o*-xylene, *p*-xylene, toluene, and methyl caprate were purchased from Sigma Aldrich. *n*-Heptadecane, *n*-tetradecene, and *n*-octadecene were obtained from K&K laboratories Inc. (Plainview, NY, USA). Methyl caproate was purchased from Alfa Aesar (Ward Hill, MA, USA). *n*-Octane, methyl myristate, indane, and 1,2,4-trimethylbenzene were purchased from Fluka. Benzene was purchased from EMD Chemicals, Inc. (Gibbstown, NJ, USA). The calibration standards were prepared in the concentration range 0.025–30 mg/mL of each analyte in methylene chloride using serial dilutions.

A mixture of three I.S.'s consisting of benzene-d_6 (I.S.1, 102 mg/mL), 2-chlorotoluene (I.S.2, 100 mg/mL), and *o*-terphenyl (I.S.3, 49.8 mg/mL) in methylene chloride was employed. Toluene-d_8 was also tested as a potential I.S. All I.S.'s were purchased from Sigma Aldrich (St. Louis, MO, USA).

For GC–FID/MS analysis, OLP samples (1.0 mL) were weighted into 2-mL autosampler vials. Then, 100.0 μL of the I.S. mixture was added and the samples were mixed.

2.2. Instrumentation

Analyses were performed using a GC–FID/MS (Agilent Technologies model 7890N GC, and 5975C MS, Santa Clara, CA, USA) equipped with an autosampler (Agilent 7386B series), a PTV, and a SS injector (Agilent). Separations were accomplished using a 100-m long DB-Petro capillary column, 0.25 mm I.D. and 0.5 μm film thicknesses (J&W Scientific, Rancho Cordova, CA, USA) coupled to a 3 m guard fused silica capillary column without any stationary phase (0.25 mm I.D.) at a constant helium flow rate of 1.5 mL min^{-1}. The performance, i.e., inertness, peak resolution, and peak shape of the column was verified weekly using diluted (2–5 ppm per analyte) Snap & Shoot 0.25 XTI test mixture (Restek, Bellefonte, PA, USA). Cryogenic cooling using liquid nitrogen was employed to ensure the separation of low boiling compounds.

Samples (0.20 μL) were injected in a split ratio of 1:30 into a multi baffle PTV liner (150 μL volume). A straight liner with a glass wool packing near the middle was used with the SS inlet. The evaluation SS and PTV injectors' performance was based on triplicate injections of a mixture of linear alkanes from *n*-pentane to *n*-octadecane at varying split ratios and injection volumes. Both the *F*-test and the Student *t*-test were used to evaluate the data obtained to a 95% confidence interval. The tested temperatures and temperature programs (of injectors and column) are listed in Table 1. The optimum injector temperature program labeled as "PTV: cold fast to 350 °C" (Table 1) was then employed for analysis of all OLP samples.

The column temperature program listed in ASTM method D6730 was used as the starting point for a series of experiments that led to the final column temperature program for OLP analysis (Table 1). Both a naphtha standard and an OLP obtained by the pyrolysis of soybean oil were used to evaluate compound separation. The final method used for analyses of OLP samples was found to be method 5 (Table 1) with a total analysis time of 133 min.

Table 1
List of methods with chromatographic experimental conditions used to evaluate the optimization. Method 5 was selected as optimum for OLP and derived fuel sample characterization.

Method name	Injector temperature program	Column temperature program
SS: 250 °C	250 °C	5 °C/2.5 °C min^{-1}/300 °C, 15 min
SS: 350 °C	350 °C	5 °C/2.5 °C min^{-1}/300 °C, 15 min
PTV: 350 °C	350 °C	5 °C/2.5 °C min^{-1}/300 °C, 15 min
PTV: cold slow to 350 °C	25 °C, 0.1 min/50 °C min^{-1}/350 °C, 5 min	5 °C/2.5 °C min^{-1}/300 °C, 15 min
PTV: cold fast to 350 °C	25 °C, 0.1 min/720 °C min^{-1}/350 °C, 5 min	5 °C/2.5 °C min^{-1}/300 °C, 15 min
PTV: cold fast to 250 °C	25 °C, 0.1 min/720 °C min^{-1}/250 °C, 5 min	5 °C/2.5 °C min^{-1}/300 °C, 15 min
1	250 °C	5 °C/5 °C min^{-1}, 50 °C, 10 min/1.5 °C min^{-1}, 200 °C, 5 min/10 °C min^{-1}, 300 °C, 15 min
2	25 °C, 0.1 min/720 °C min^{-1}/350 °C, 5 min	5 °C, 10 min/2.5 °C min^{-1}/300 °C, 15 min
3	25 °C, 0.1 min/720 °C min^{-1}/350 °C, 5 min	5 °C/1.5 °C min^{-1}, 50 °C/3 °C min^{-1}, 300 °C, 15 min
4	25 °C, 0.1 min/720 °C min^{-1}/350 °C, 5 min	5 °C/2 °C min-1, 100 °C/3 °C min^{-1}, 300 °C, 15 min
5	25 °C, 0.1 min/720 °C min^{-1}/350 °C, 5 min	5 °C/2.5 °C min^{-1}/300 °C, 15 min
6	25 °C, 0.1 min/720 °C min^{-1}/350 °C, 5 min	5 °C/3 °C min^{-1}/300 °C, 15 min
7	25 °C, 0.1 min/720 °C min^{-1}/350 °C, 5 min	5 °C/5 °C min^{-1}/50 °C/3 C min^{-1}, 300 °C, 15 min

PTV: cold fast to 350 °C injector temperature program was employed for methods 1 through 7.

The GC column was connected to MS and FID detectors through a two-way splitter with a makeup gas (helium at a constant pressure of 26.2 kPa) using connecting capillaries without a stationary phase. The dimensions of the connecting capillaries (0.18 mm I.D.), 3.55 m long to MS and 0.65 m long to FID, defined the split flow ratio of 1:2 (MS:FID) and ensured that the flow rates to the MS detector did not disrupt the vacuum diffusion pump operation while minimizing the peak broadening caused by void volumes in the splitter. Thus, at the lowest (5 °C) and highest temperature (300 °C), the maximum flow rates into the MS were 2.0 mL min^{-1} and 0.6 mL min^{-1}, respectively. At 5 °C the linear velocities for FID and MS were 393 cm min^{-1} and 196 cm min^{-1}, respectively. At 300 °C the linear velocities reached 118 cm min^{-1} at the FID and 59 cm min^{-1} at the MS. With the 0.18 mm I.D. capillaries installed to the detectors, a constant retention time offset of 0.04 min between the MS and FID signal was observed. The FID temperature was set to 350 °C and that of the MS transfer line to 280 °C. The MS data were acquired using an electron ionization (EI) of 70 eV in a full scan mode (35–500 *m/z*) at a scan rate of 2.83 scan s^{-1}.

The instrumentation and method for the analysis of acids and alcohols in OLP samples selected for this study was reported previously [7]. This method employed BSTFA (100 μL) derivatization with 20 μL of OLP at 60 °C for 1 h. A recovery standard mixture

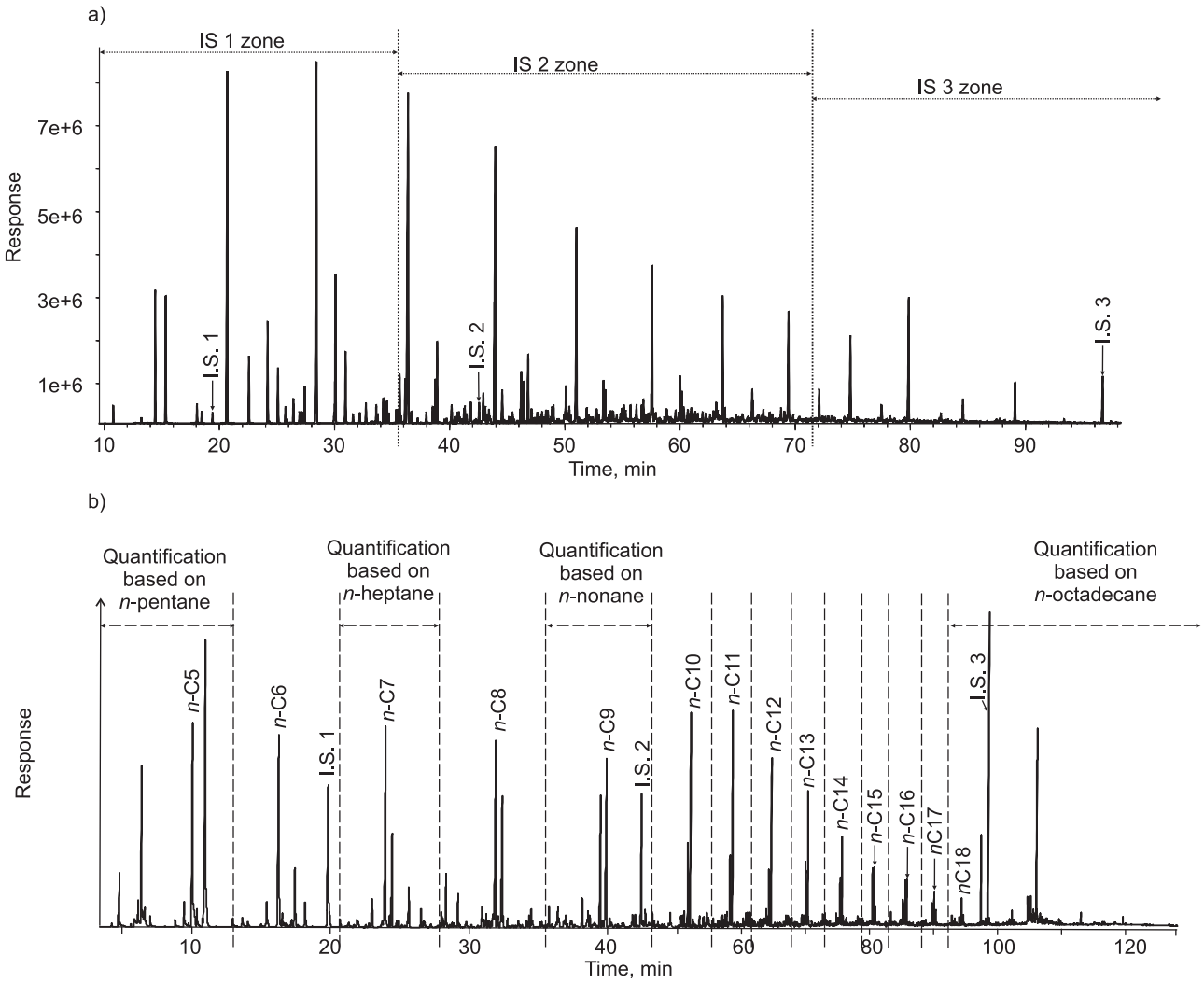

Fig. 1. (a) A soybean OLP GC–FID chromatogram demonstrating I.S. zones used for quantification, (b) a soybean OLP GC–FID chromatogram with retention ("quantification") windows.

consisting of deuterated acetic, butyric, nonanoic, and hexadecanoic acid was added prior to sample derivatization to correct for reaction efficiency. After derivatization, the samples were diluted to 1.0 mL using dichloromethane and then 5.0 μL of an I.S. (*o*-terphenyl, 50.34 mg/mL) was added.

2.3. Identification method

Analyte identification was based on matching retention times and mass spectra to standards. If no standard was available, the tentative identification was performed by matching the mass spectra to the standard reference mass spectra of the National Institute of Standards and Technology (NIST) library, version 05. The required match with the reference mass spectrum was 80% and confirmed visually for the major ions present.

2.4. Quantification method

As opposed to ASTM method D 6730, samples were quantified using the I.S. method. The application of three I.S.'s of different volatilities: I.S.1 (b.p. 79 °C), I.S.2 (b.p. 159 °C), and I.S.3 (b.p. 332 °C) was evaluated on a series of linear $C_{5–18}$ *n*-alkanes. The I.S.'s were applied to analytes eluting within their retention time zones. The starting and ending points of these zones were set at mid-points between the retention times of two adjacent I.S.s' (Fig. 1a).

Although FID provides a response proportional to the number of carbon atoms, its response is influenced by discrimination at the injection port and the presence of heteroatoms. Therefore, in this study a calibration standard was designed that contained a full set of linear alkanes along with selected compounds representing different classes of chemicals (alkenes, aromatic compounds, and FAMEs). Table 2 provides the list of standards, slopes (k), regression coefficients (R^2), standard errors (s_y) of the predicted y-value for each x-value, upper calibration ranges, and limits of quantification (LOQs) using the FID response. The set of calibration standards (providing a six-point curve over the concentration range) was analyzed at the beginning and end of each sequence. A middle calibration point was analyzed after every 10 samples. A full calibration range was analyzed whenever a middle calibration point deviated from the previous analysis by more than 5%. Otherwise no action was taken. The instrumental LOQs were calculated from the calibration curves generated using a least square linear regression that was forced through an origin using the following equation [36]:

$$\mathrm{LOQ} = \frac{10 \times s_y}{k}$$

The quantification of non-calibrated species was based on the RFs of the nearest alkane standard and that of a calibration standard representing that specie's particular class of compounds. To assign appropriate response factors to analytes for which calibration

Table 2
Composition of the calibration standard mixture and GC–FID least square linear curve calibration parameters, where R^2 is a square correlation coefficient and s_y is the standard error. The LOQs are expressed per 0.2 μL injection.

Name	Formula	MW [g mol^{-1}]	Upper conc. [mg/mL]	Slope	R^2	s_y	LOQ [μg]
n-Pentane	C_5H1_2	72.15	27	0.067	1.000	0.005	6
n-Hexane	C_6H_{14}	86.21	27	0.085	1.000	0.001	3
n-Heptane	C_7H_{16}	100.23	27	0.090	1.000	0.002	2
n-Octane	C_8H_{18}	114.25	27	0.115	1.000	0.007	4
n-Nonane	C_9H_{20}	128.27	30	0.115	1.000	0.010	1
n-Decane	$C_{10}H_{22}$	142.29	28	0.119	1.000	0.008	2
n-Undecane	$C_{11}H_{24}$	156.31	29	0.118	1.000	0.010	7
n-Dodecane	$C_{12}H_{26}$	170.33	27	0.119	1.000	0.011	15
n-Tridecane	$C_{13}H_{28}$	184.36	27	0.120	1.000	0.010	5
n-Tetradecane	$C_{14}H_{30}$	198.39	30	0.169	1.000	0.015	1
n-Pentadecane	$C_{15}H_{32}$	212.41	27	0.166	1.000	0.014	4
n-Hexadecane	$C_{16}H_{34}$	226.44	29	0.166	1.000	0.014	3
n-Heptadecane	$C_{17}H_{36}$	240.47	27	0.168	1.000	0.013	0.9
n-Octadecane	$C_{18}H_{38}$	254.49	27	0.174	1.000	0.014	3
1-Hexene	C_6H_{12}	84.16	27	0.086	1.000	0.002	2
1-Nonene	C_9H_{18}	126.24	29	0.114	1.000	0.008	2
1-Tetradecene	$C_{14}H_{28}$	139.70	26	0.133	1.000	0.011	7
1-Octadecene	$C_{18}H_{36}$	252.47	26	0.162	1.000	0.012	3
Benzene	C_6H_6	78.11	30	0.097	1.000	0.008	20
Toluene	$C_6H_5CH_3$	92.14	31	0.098	1.000	0.006	3
p-Xylene	$C_6H_4(CH_3)_2$	106.17	31	0.124	1.000	0.010	8
Naphthalene	$C_{10}H_8$	128.17	30	0.132	1.000	0.011	2
Indane	C_9H_{10}	118.18	28	0.125	1.000	0.010	2
1,2,4-Trimethylbenzene	$C_6H_3(CH_3)_3$	120.19	27	0.124	1.000	0.009	0.7
Methyl propionate	$C_2H_5CO_2CH_3$	88.10	29	0.036	1.000	0.003	79
Methyl hexanoate	$C_5H_{11}CO_2CH_3$	130.18	29	0.072	1.000	0.005	6
Methyl decanoate	$C_9H_{19}CO_2CH_3$	186.29	30	0.088	1.000	0.009	21
Methyl myristate	$C_{13}H_{25}CO_2CH_3$	242.40	28	0.135	1.000	0.010	7
Methyl stearate	$C_{17}H_{33}CO_2CH_3$	298.50	26	0.131	1.000	0.008	15

standards were not available, the retention time ranges were created pertaining to different alkane standards. These retention windows were defined by the intervals shown in following equation

Quantification interval

$$Z = t_{RAA(X-1)} + t_{RAA(X)} - \frac{t_{RAA(X-1)} - t_{RAA(X-1)}}{2} \quad \text{min} \quad \text{to}$$

$$t_{RAA(X-1)} + \frac{t_{RAA(X+1)} - t_{RAA(X)}}{2} \quad \text{min}$$

e.g.,

$$\text{Quantification interval} \quad 1 = 0 \quad \text{min} \quad \text{to} \quad \frac{t_{RAA06} - t_{RAA05}}{2} \quad \text{min}$$

where Z represents a number, AA is linear alkane, and X is a number of carbon atoms.

A sample chromatogram with the quantification intervals is shown in Fig. 1b. The RFs (slope) of non-calibrated compounds were derived from the RFs representative for a particular class of compounds with respect to the quantification interval of the nearest alkanes. For branched alkanes and cycloalkanes, the RFs were assigned as those of linear alkanes in the same quantification interval. For other classes of compounds (e.g., alkenes), the RF of an alkane eluting within the particular quantification interval was corrected by a factor obtained from the standard with the appropriate functionality (e.g., the ratio of the nearest eluting alkene and alkane from the calibration standard).

$$\text{RF}_{\text{heptene}} = \frac{\text{RF}_{n\text{-heptane}}}{\text{RF}_{n\text{-nonane}}} \times \text{RF}_{\text{nonene}}$$

It is noteworthy that in OLPs derived from canola or soybean oil, broad peaks of fatty acids (FAs) co-eluted with hydrocarbons. The hydrocarbons (coincidentally represented by small peaks) were then quantified using only the peak areas on top of the non-derivatized FA peak (see Fig. 2). Also, methyl acetate co-eluted

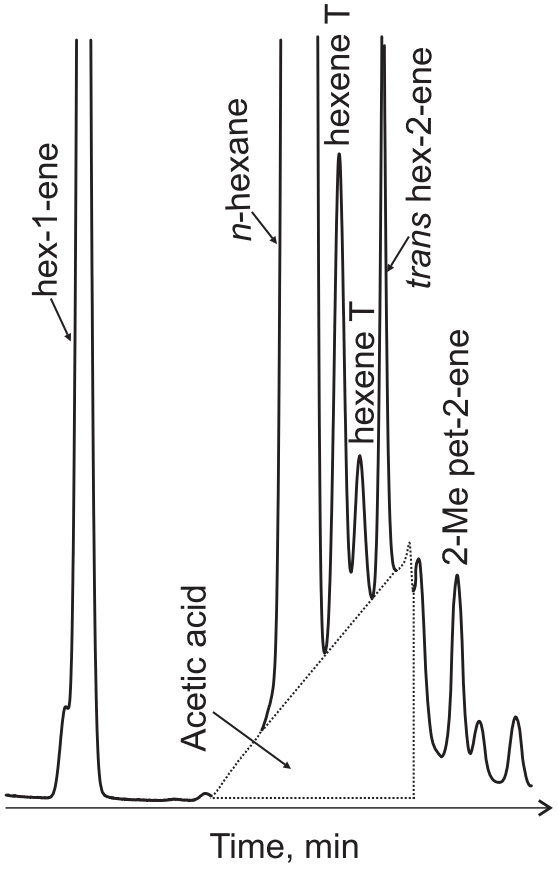

Fig. 2. An example of underivatized acetic acid co-elution with alkane and alkenes in soybean and canola OLP.

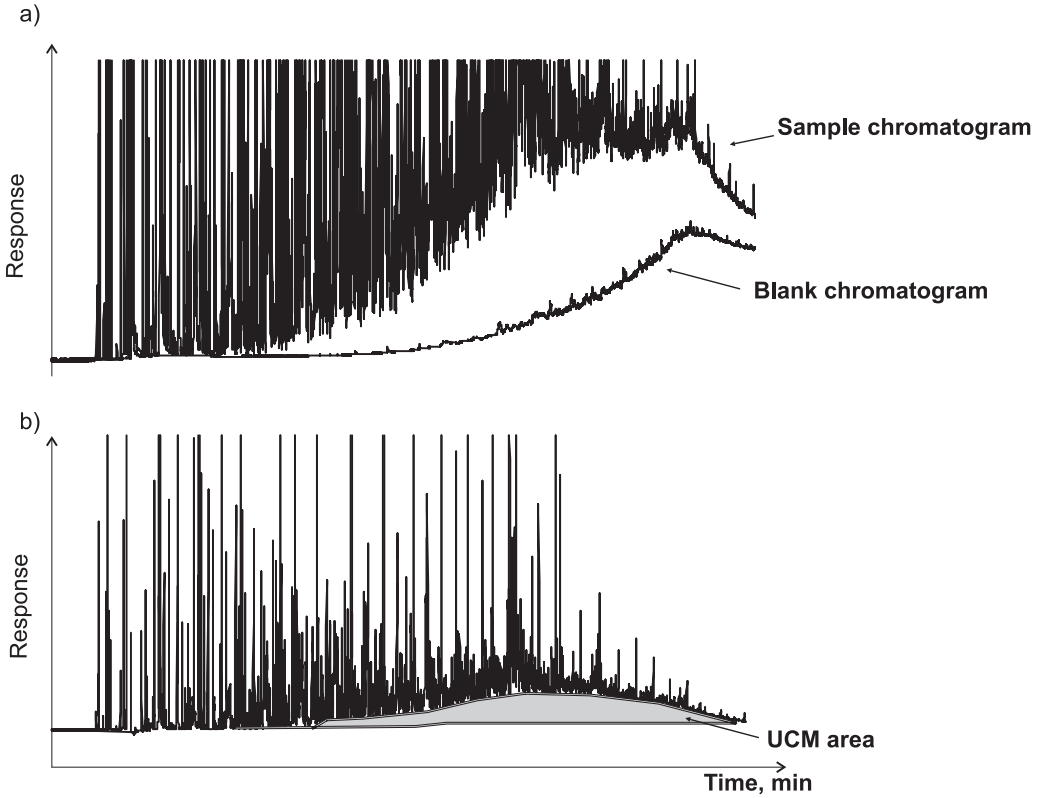

Fig. 3. (a) GC–FID chromatogram of an OLP analysis overlaid with a blank GC–FID chromatogram obtained by analyzing solvent, (b) GC–FID chromatogram after subtraction of the blank chromatogram from the sample chromatogram that was integrated along the peak bases. The shaded area represents the UCM.

with methylene chloride (employed solvent for I.S. mixture) thus making its identification and quantification impossible.

Besides hydrocarbons and esters, the concentrations of polar species [7], unidentified peaks, and UCM were estimated to close the mass balance. The unidentified peaks, from which the non-derivatized FAs were subtracted, were quantified using a RF of a representative calibrated compound, namely *n*-decane. The UCM area was determined by subtracting the area of all peaks and a blank-solvent chromatogram from the total area obtained by integrating a sample chromatogram along the baseline (Fig. 3). Based on the nature of the UCM (using the representative background mass) and the retention in the chromatogram (in the near proximity of *n*-heptadecane), the abundance of UCM was estimated using the RF of the nearest linear alkane.

3. Results and discussion

3.1. Optimization of GC parameters

To achieve high accuracy in quantitation, we evaluated the temperature (on the SS and PTV inlets), the temperature gradient of the PTV (to minimize compound discrimination), and the GC oven temperature program (derived from ASTM D 6730 method).

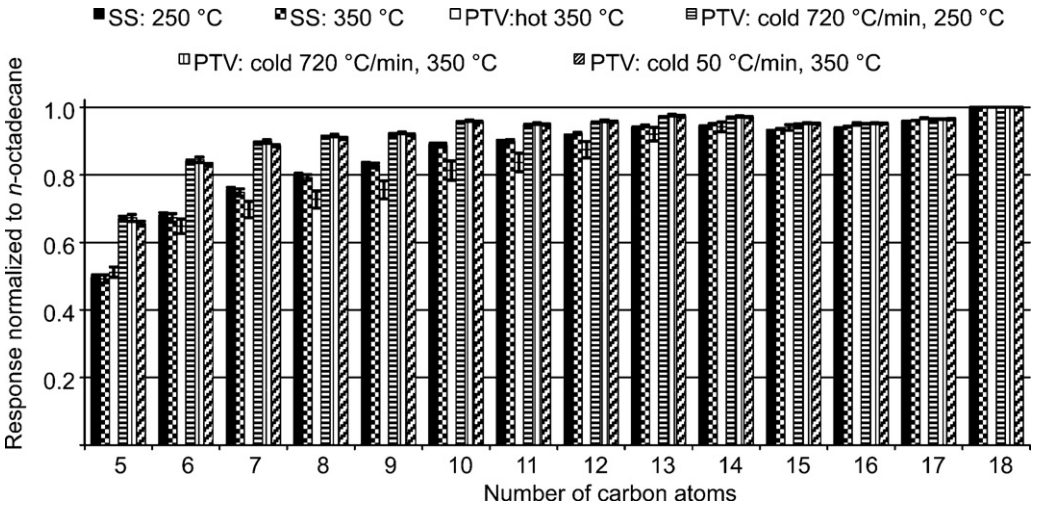

Fig. 4. The effect of injector (SS or PTV) and injection method on compound discrimination. The data are presented as a mean ± SD ($n = 3$).

Table 3
The resolution of critical pairs of compounds analyzed using GC–FID/MS with different column temperature programs (details are provided in Table 1). The resolution was calculated from the reconstructed ion chromatograms using a specific ion for a particular compound.

				Method name						
				1[a]	2	3	4	5	6	7
Factors optimized										
Isothermal period at 5 °C [min]				10	10	0	0	0	0	0
1st temperature gradient [°C min^{-1}]				5	2.5	1.5	2	2.5	3	5
Isothermal period at 50 °C [min]				50	0	0	0	0	0	0
2nd temperature gradient [°C min^{-1}]				1.5	2.5	3	3	2.5	3	3
Compound 1	*m/z*	Compound 2	*m/z*	Resolution (R_s)						
Benzene-d_6	84	Methylcyclopentene	82	1.3	1.6	2.0	1.6	1.1	0.9	0.03
Methylcyclopentene[b]	82	Benzene[b]	78	0.6	0.3	0.3	0.5	0.8	0.9	1.3
n-Propylcyclopentane	69	*n*-Ethylcyclohexane	82	0.2	1.5	1.4	1.4	1.7	1.9	2.0
2-Chlorotoluene	126	*n*-Propylbenzene	120	8.1	3.4	0.02	3.5	2.9	2.5	2.6
Toluene-d_8	98	1,5-Dimethylcyclopentane	96	0.7	0.2	0.6	0.2	0.1	0.5	1.1
Toluene-d_8	98	*n*-Ethylcyclopentane	96	2.0	1.5	2.3	1.8	1.4	0.9	0.2
n-Methylcycloheptane	97	*m*-Xylene	91	5.1	1.4	1.7	2.0	1.1	0.7	0.4
m-Xylene	91	*p*-Xylene	91	1.6	0.9	1.0	1.1	0.9	0.9	0.8
1-Methylnaphthalene[b]	142	*n*-Tridecane[b]	57	16	5	3.9	2.0	5.6	3.6	3.5
n-Butylcyclopentane	69	*n*-Propylcyclohexane	82	6.0	2.1	2.1	2.3	1.9	1.6	1.4
n-Pentylcyclopentane	69	*n*-Butylcyclohexane	82	4.4	1.7	1.7	1.9	1.7	1.4	1.3
n-Hexylcyclopentane	69	*n*-Pentylcyclohexane	82	3.6	1.5	1.3	1.6	1.5	1.2	1.1
n-Heptylcyclopentane	69	*n*-Hexylcyclohexane	82	2.0	0.5	0.4	0.5	0.6	0.3	0.3
n-Octylcyclopentane	69	*n*-Heptylcyclohexane	82	1.1	0.1	0.05	NS[c]	0.08	0.06	0.06
n-Nonylcyclopentane	69	*n*-Octylcyclohexane	82	0.1	0.4	0.6	0.6	0.4	0.6	0.6
n-Decylcyclopentane	69	*n*-Nonylcyclohexane	82	0.4	1.0	1.3	1.3	1.2	1.3	1.3
n-Undecylcyclopentane	69	*n*-Decylcyclohexane	82	1.0	1.3	1.7	NS	1.3	1.6	1.6
I.S.3 Retention time	[min]		182	107	98	99	97	87	77	

[a] Denotes ASTM D 6730 method temperature program.
[b] Denotes pairs of compounds evaluated in ASTM D6730 method.
[c] "NS" denotes not separated, Shaded area represent R_s < 0.75 (50%).

3.1.1. The effect of injection conditions on compound discrimination

An important step in developing an accurate method for the analysis of OLPs was to minimize discrimination at the injection port. This step enabled an accurate quantification with only a limited set of standards. We evaluated the injection conditions for a series of *n*-alkanes (C_{5-18}) using two injectors on the same instrument: (1) a classical SS injector and (2) a PTV using the temperature programs listed in Table 1.

A comparison of hot injections with both injectors and cold injection with the PTV is shown in Fig. 4. Hot injection methods for both injectors exhibited a more pronounced discrimination of lower-MW *n*-alkanes (C_{5-9}) than cold injection techniques with the PTV (Fig. 4). This discrimination of more volatile species in our study may seem to be in contrast to previously reported discrimination toward higher boiling species. However, the previous studies targeted species with higher carbon numbers (i.e., $\geq C_8$). The discrimination and higher standard deviation of highly volatile species for hot split injection may perhaps be explained by differences in diffusion speeds where heavier species prevent the smaller ones from entering the column [37]. A decreased discrimination of C_{5-7} volatile alkanes was observed for all cold split PTV injection techniques (Fig. 4). It was also verified that the final PTV temperatures of 250 or 350 °C, using 0.2 μL injections with split ratios of 1:30 and 1:100, and 0.5 μL injections with a 1:100 split ratio, did not significantly affect the linear alkanes' responses (data not shown).

Table 4
Resolution of selected pairs of compounds employing the optimized GC–MS/FID method (method 5, Table 1) applied to canola, soybean, and CME OLP.

Compound 1	*m/z*	Compound 2	*m/z*	Resolution		
				Canola	Soybean	CME
Benzene-d_6	84	Methylcyclopentene	82	1.2	1.2	1.2
Methylcyclopentene[a]	82	Benzene[a]	78	0.7	0.7	0.8
n-Propylcyclopentane	69	*n*-Ethylcyclohexane	82	1.7	1.8	1.8
2-Chlorotoluene	126	*n*-Propylbenzene	120	2.9	2.9	2.8
Methylcycloheptane	97	*m*-Xylene	91	1.0	1.1	1.0
m-Xylene	91	*p*-Xylene	91	1.0	0.9	0.9
1-Methylnaphthalene[a]	142	*n*-Tridecane[a]	57	5.2	5.3	5.2
n-Butylcyclopentane	69	*n*-Propylcyclohexane	82	2.0	2.2	2.0
n-Pentylcyclopentane	69	*n*-Butylcyclohexane	82	1.7	1.7	1.7
n-Hexylcyclopentane	69	*n*-Pentylcyclohexane	82	1.4	1.5	1.4
n-Heptylcyclopentane	69	*n*-Hexylcyclohexane	82	0.6	0.6	0.5
n-Octylcyclopentane	69	*n*-Heptylcyclohexane	82	0.01	NS[b]	0.04
n-Nonylcyclopentane	69	*n*-Octylcyclohexane	82	0.5	0.5	0.5
n-Decylcyclopentane	69	*n*-Nonylcyclohexane	82	1.1	1.2	1.3
n-Undecylcyclopentane	69	*n*-Decylcyclohexane	82	3.0	2.9	2.7

[a] Denotes pairs of compounds evaluated in the ASTM method D6730.
[b] "NS" denotes not separated

Table 5
The composition of OLP generated by the pyrolysis of soybean oil, canola oil and CME. The sample replicates were generated by three independent pyrolysis experiments. For the GC–FID/MS analysis replicates, a single sample was analyzed within a 24 h, in random order (in between other samples). All results are expressed as a mean and SD.

	Compound	Soybean pyrolysate		CME pyrolysate		Canola pyrolysate		Canola pyrolysate		JP-8	
		Average (wt%)	SD	Average (wt%)	SD	Average (wt%)	SD	Average (wt%)	SD	Average (wt%)	SD
Alkanes	$C_{3–6}$ linear	4.5	0.9	3.1	0.9	4.6	0.4	4.38	0.02	0.04558	0.00009
	$C_{7–10}$ linear	5	1	3	2	9.4	0.5	9.203	0.008	3.70	0.05
	$C_{11–17}$ linear	6	1	2.1	0.3	5.6	0.4	5.28	0.02	8.13	0.03
	$C_{18–26}$ linear	0.63	0.01	0.25	0.03	0.83	0.08	0.838	0.008	0.0144	0.0002
	Total linear	16	3	9	2	20.4	1.4	19.70	0.04	11.89	0.08
	Total branched	0.5	0.2	0.4	0.2	0.61	0.07	0.568	0.002	17.89	0.06
	Total cyclo	3.6	0.9	3.4	0.5	4.8	0.4	4.563	0.023	7.16	0.05
	Total	20	4	13	1	25.8	1.6	24.84	0.05	36.9	0.2
Alkenes	Total linear terminal	2.2	0.1	2.3	0.5	2.3	0.2	2.011	0.004	–	
	Total branched terminal	0.23	0.05	0.28	0.05	0.23	0.04	0.216	0.002	–	
	Total linear non-terminal	3.8	0.9	0.06	0.01	4.4	0.3	4.196	0.005	–	
	Total branched non-terminal	0.05	0.01	2.0	0.4	0.055	0.001	0.0477	0.0002	–	
	Total cyclo	1.5	0.2	1.7	0.2	1.5	0.3	1.331	0.003	–	
	Total	8	1	6	1	8.5	0.7	7.803	0.009	–	
Aromatics	Total BTEX	0.9	0.3	0.9	0.1	0.97	0.07	0.903	0.004	10.431	0.047
	Total alkylbenzenes	1.6	0.5	1.3	0.1	1.7	0.1	1.522	0.006	11.64	0.05
	Total	2.5	0.9	2.2	0.3	2.7	0.2	2.425	0.006	1.40	0.01
Polyaromatics	Total indanes and indenes	1.0	0.2	0.9	0.1	1.1	0.1	1.062	0.003	4.137	0.003
	Total naphthalenes and fluorenes	0.7	0.2	0.40	0.09	0.83	0.05	0.781	0.005	5.54	0.01
	Total	1.6	0.3	1.3	0.2	2.0	0.2	1.843	0.006	54.11	0.24
Ketones	Total	0.17	0.05	0.008	0.003	0.3	0.1	0.348	0.004	–	
Fatty acids[a]	$C_{2–3}$	2.6	0.2	1.09[c]	0.06	2.2	0.8	2.1	–[d]	–	
FAMEs[b]	$C_{4–9}$	7.8	0.8	8.2	0.5	5.8	1.1	5.3	–[d]	–	
	$C_{10–14}$	3.3	0.3	2	1	3.3	0.6	3.0	–[d]	–	
	$C_{15–16}$	2.8	0.3	1.3	0.8	0.58	0.09	0.62	–[d]	–	
	$C_{17–18}$	1.4	0.2	1.2	0.7	0.6	0.1	0.6	–[d]	–	
	$C_{19–24}$	–		0.4	0.1	–		–		–	
	Total linear saturated	18	1	14	3	12	3	11.65	–[d]	–	
	Total branched saturated	–		0.14	0.02	–		–		–	
	Total saturated	17.8	1.1	15	3	12	3	–		–	
	Total unsaturated	0.3	0.1	4	1	0.21	0.02	0.23	–[d]	–	
	Total	18	1	18	2	13	3	12	–[d]	–	
Difatty acids	$C_{4–9}$	0.27	0.03	0.24	0.02	0.2	0.1	–		–	
	$C_{10–14}$	0.5	0.1	1.2	0.3	0.16	0.08	0.2	–[d]	–	
	Total	0.8	0.2	1.5	0.3	0.3	0.2	0.2	–[d]	–	
Methyl esters	Total	–		0.40	0.06	–		–		–	
	Total identified	51	7	40	2	52	4	49.49	0.06	54.1	0.2
	Total unidentified	10	1	15	2	17	1	13.76	0.09	21.33	0.05
	Total unresolved	37	4	19	3	25	3	27.8	0.8	28.7	0.7
	Total	99	3	77	2	94	2	91.1	0.8	104.2	0.5

[a] Present in biofuels generated from soybean and canola oil.
[b] Present in biofuels generated from CME.
[c] Denotes only methyl propionate, methyl formate was not detected.
[d] "–" denotes single analysis.

3.1.2. Evaluation of column temperature program

The "PTV: cold fast to 350 °C" program was used for the column temperature optimization because it minimized the discrimination of the low-MW analytes and ensured effective transfer of high-MW compounds onto the column.

The column temperature program was evaluated using a naphtha standard (low boiling point distillation fraction of crude petroleum) and soybean oil OLP. The evaluation of separation efficiency was based on the resolution (R_s) of three groups of analytes: (1) two pairs of compounds, relevant to both OLPs and petroleum analysis (i.e., used in ASTM method D 6730); (2) pairs of potential I.S.s' and neighboring compounds; and (3) pairs of compounds critical for an effective separation of OLP analytes. The aim of this temperature program evaluation was to achieve a shorter analysis time (expressed by the retention time of I.S.3 Table 3) using a single temperature gradient, which would enable a more accurate prediction of retention times for a homologous series of compounds.

The temperature programs (methods 2–7, Table 1) were derived from ASTM method D6730 (method 1, Table 1). The D6730 isothermal period of 10 min at 5 °C (methods 1 and 2, Table 3) turned out to be excessive for separation of volatile OLP components. Similarly, the 50 min hold at 50 °C in this method (method 1, Table 3) was too long, resulting in a $R_s > 6$ for the pair 2-chlorotoluene and *n*-propylbenzene. Furthermore, this extensive isothermal period caused co-elution of *n*-propylcyclopentane and *n*-ethylcyclohexane ($R_s < 0.3$, method 1, Table 3).

Pursuing the goal of developing a single linear gradient method, we then eliminated the isothermal periods at 5 and 50 °C and evaluated the impact of first temperature gradients in the range of 1.5–5 °C min^{-1} (methods 3–7, Table 3). ASTM D6730 states that the separation of a critically important pair, 1-methylcyclopentene and benzene, is based on column selectivity and that only minor variance is observed with changing the column temperature program. However, in our work we found that benzene and 1-methylcyclopentene co-eluted when using the slower of these temperature gradients (methods 3 and 4, Table 3). Whereas the faster temperature gradient of 5 °C min^{-1} negatively influenced the separation of the neighboring pairs methylcyclopentene/benzene-d_6 ($R_s = 0.03$, Table 3) and *n*-methylcycloheptane/*m*-xylene ($R_s = 0.4$, Table 3). The preferred method was found to be using a linear 2.5 °C min^{-1} gradient (method 5, Table 3). This method provided a good separation of the majority of the targeted compound pairs while also shortening the total analysis time from 190 min (the time required for ASTM D6730) to 133 min.

When selecting I.S.'s, it was not possible to separate toluene-d_8 (a potential I.S.) from the neighboring eluting compounds. Benzene-d_6, 2-chlorotoluene, and *o*-terphenyl were separated from the nearest-eluting components (method 5, Table 3) and therefore these three standards were employed in further analysis.

Closely eluting *n*-alkyl cyclopentanes and *n*-alkyl cyclohexanes were selected as target compounds for OLP analysis because their elution spreads along the entire elution range of the analytes. Unfortunately, at least one of these pairs remained unresolved for all evaluated methods. Separation of *n*-octylcyclopentane and *n*-heptylcyclohexane was limiting for the final method 5.

The efficient separation of the majority of the targeted compounds, as listed in Table 3 using method 5 was confirmed using samples generated from each of the three reference feedstocks: canola oil, soybean oil, and CME (Table 4). The separation of individual species of CME OLP is also shown on a sample GC–FID chromatogram provided in supplementary materials (Fig. S3).

3.2. Quantification of OLPs

The developed method was applied to several representative samples produced by the pyrolysis of TGs or CME, as summarized in Table 5 (Table S3 provides detailed compositions in supplementary material). Over 250 compounds were identified and quantified in OLPs using GC–FID/MS. The significance of the actual composition of comparable OLPs obtained through the application of the analytical methods discussed herein have been provided in detail elsewhere [7,13,38,39]. Thus the present discussion is limited to method development implications.

In contrast to previously used techniques for OLP analysis [2,16,17,22], in this work the mass balance was assessed by estimating the overall concentration of UCM and unidentified components while using the representative RFs related to the actual mass of analytes. Mass balance closure was obtained for soybean and canola oil OLPs, indicating that all species were GC-elutable (Table 5). The accuracy of quantification was confirmed using a petroleum jet fuel (JP-8) for which all species (ca. 100 wt%) were GC-elutable. By contrast, incomplete mass balance in CME OLP indicated the presence of GC-non-elutable (high-MW, nonvolatile, and/or thermally unstable) species.

When estimating the mass balance, the abundance of unidentified compounds and UCM were evaluated individually. Extraction of common ions characteristic for various classes of compounds were used to identify the nature of each of these categories. The unidentified components appeared to consist of alkanes, alkenes, polyaromatics, and FAMEs (for CME). The RF of *n*-decane was selected as an average representative for quantification.

Based on characteristic ions, the UCM was fairly diverse in its composition. For example in CME OLP, FAMEs, alkenes, and alkanes seemed to have similar contributions to the UCM. In this study, UCM quantification was reported using the RF of *n*-heptadecane. However, using this RF we were not able to achieve complete mass closure for CME. We then attempted to quantify the UCM using RFs representing alkenes and FAMEs. UCM quantification using the RFs of *n*-octadecene or methyl heptadecanoate resulted in ca. 1 or 5 wt% increase of the UCM, respectively. This improved the mass balance from 77 wt% to 82 wt%, but was still incomplete. While this approach has provided some insight into UCM components, we were not able to completely quantify the UCM. It is possible that the difficult to characterize (by GC) UCM species may be similar to asphaltenes which are found when processing petroleum [40,41].

GC–FID/MS analysis repeatability was based on five analyses of a single canola OLP and a triplicate measurement of a petroleum derived jet (JP-8) fuel (Table 5 and Table S4 in supplementary material). An RSD below 5% was obtained for all individual analytes. It should be noted that compositional variations among the replicate OLP samples analyzed (sample replicates in Table 5) did not significantly affect the overall mass balance. The differing composition of OLPs derived from various crop oils and resulting mass balance confirmed our original hypothesis that in contrast to petroleum products, OLPs have different constituents and not all species may be GC-elutable.

4. Conclusions

The developed method enabled quantitative characterization of three types of biofuel intermediate products generated by the pyrolysis of soybean oil, canola oil, and CME. The method's performance was verified on a sample of JP-8 fuel. The use of a PTV limited compound discrimination at the injector and improved reproducibility of the injection method. Accurate identification (using FID and MS) and quantification (using the designed calibration standards) of individual species was achieved. The quantification of individual hydrocarbons, unidentified and polar organic species, and UCM allowed the most accurate assessment of the mass balance for these types of organic mixtures. This is in contrast to typically reported results on the composition of biofuels using methods with

quantitation based on normalization of total area. The developed method demonstrated the importance of having an extremely accurate analytical method for renewable fuels in various stages of their development.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2011.12.013.

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