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Quantitative Analysis of Saturated Monoglyerides in Palm Oil Biodiesel by Gas Cromatography-Mass Spectrometry

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ABSTRACT

The current issue in biodiesel implementation in Indonesia is fuel filter clogging caused by precipitates. Some studies show that the saturated monoglycerides (SMGs) contribute the formation of precipitates in biodiesels. This research used the GC-MS method with the selected ion monitoring (SIM) approach to quantify SMG in palm oil biodiesel. Monomyristin (1-C14:0), monopalmitin (1-C16:0), monostearin (1-C18:0) and monoarachidin (1-C20:0) were selected as model compounds. The analysis was carried out using thermo scientific GC-MS equipped with a 5% phenyl methyl siloxane column (30 mx0.25 mmx0.25 µm). The fragmentation ion of certified reference material (CRM) of SMGs was determined by utilizing a scanning approach. Then, the limit of detection (LOD) and limit of quantitation (LOQ) of each CRM was calculated by SIM to obtained SMG content at concentration 0.1, 0.05 and 0.025 µg mL^{-1} with S N^{-1} ratio 3 and 9, respectively. Linearity of this method was determined at a concentration of 0.1-1.0 mg mL^{-1}. The SMG content in palm oil-based biodiesels were analyzed through silylation with MSTFA and utilizing a monononadecanoin (1-C19:0) as in internal standard (IS). The results showed that the quantitation ion of 1-C14 ions for 1-C16:0, 1-C18:0, 1-C19:0 and 1-C20:0 were 357, 371, 399, 413 and 427 respectively. The LODs of this method were 1.48 ppb-23.97 ppb and LOQs were 4.43 ppb-71.92 ppb. This method showed a highly correlative response indicated by very high linearity with r=0.996-0.998. The highest SMG content in palm oil based biodiesel was monopalmitin (1-C16:0). Among palm based biodiesels, stearin biodiesel contained the highest SMG (0.333%-mass).

Keywords: biodiesel, GC-MS, monopalmitin, SIM, SMG

INTRODUCTION

Biodiesel is usually produced through transesterification. This is a sequence of three reversible reactions, in which triglyceride molecules are converted step by step into diglyceride, monoglyceride and finally glycerol plus three alkyl esters. The presence of monoglyceride in biodiesel is due to an incomplete conversion in the last transesterification reaction. Monoglyceride may cause formation of

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precipitates in biodiesel (Rapaka 2012; Plata 2015; Girry 2018) and therefore, the Indonesian biodiesel standard (SNI 7182:2015) specifies that its content must be limited to a maximum of 0.8%-mass (BSN 2015). Other parameters that also relate to the formation of precipitates are cloud point and pour point. Generally, pour point is lower than cloud point (Knothe & Dunn 2009; Chupka 2011).

In Indonesia, most biodiesel is produced from palm oil (Afriyanti *et al*. 2016). The fatty acids that compose palm oil and its biodiesel are myristic, palmitic, stearic and oleic acids (Table 1), of which palmitic and oleic acids are the major ones (Gunstone 2004; Hasibuan 2012). Monoglyceride of saturated fatty acids such as myristic, palmitic, stearic and arachidic acids have a much greater tendency to form precipitates than that of unsaturated fatty acids.

Table 2 shows the melting point of saturated monoglycerides, SMG (Vereecken 2009). The longer the carbon chain, the higher the tendency of an SMG to form precipitates. The proportion of SMG in the MGs that contaminates a biodiesel becomes higher with increasing saturated fatty acid content of the vegetable oil raw material. Imperfection in the production process is also a factor that influences the MGs content of a biodiesel. The two steps transesterification process will usually result in lower MGs content (Rapaka 2012).

MG or better, SMG content is an important quality parameter of biodiesel, especially for operations at low temperatures, and therefore should be quantified by a sensitive and accurate method. In SNI 7182:2015, the MG content is analyzed using the AOCS Cd 11-57 method which has now been withdrawn by the AOCS, probably because it could only measure α-mono-glyceride (AOCS 2018). The standard methods currently in use are EN14105 or ASTM D6584 for simultaneous determination of mono-, di-, and triglyceride content of biodiesel (European Standard 2011; ASTM 2015). These methods, however, can not analyze the SMG content, which has been identified as the main component causing precipitates in biodiesel.

Yang *et al*. (2011) reported a simultaneous characterization and determination of oxygenated polar impurities-free fatty carboxylic acids (FFAs), partial glycerides, residual glycerol and free sterols in various biodiesels based on the combination of solid-phase extraction (SPE), silylation and GC MS^{-1} technologies. This research suggests that FFA, glycerol and MGs have great potential as target compounds for forensic identification of biodiesels and for future research on the degradation of biodiesels.

Table 2 Saturated monoglyerides melting point

SMG	Symbol	Melting point (°C)
Monomyristin	1-C14:0	72.4
Monopalmitin	1-C16:0	78.0
Monostearin	1-C18:0	82.7
Monoarachidin	1-C20:0	86.1

Table 1 Fatty acid composition of crude palm oil, stearin and olein

Fatty acid	Palm oil		Palm olein		Palm stearin	
	Gunstone, 2004	Hasibuan, 2012	Gunstone, 2004	Hasibuan, 2012	Gunstone, 2004	Hasibuan, 2012
Myristic	0	1-2	0	0.86-1.21	0	1.04-1.37
Palmitic	44	48-74	41	39.30-42.55	47-74	57.30-66.07
Stearic	4	3.9-6	4	3.65-4.44	4-6	4.19-5.35
Oleic	39	15.5-36	31	40.48-44.11	16-37	22.09-29.89

Hischegger (2014) developed a new method for the quantification of SMGs in biodiesels from waste animal fats and used cooking oil using GC-MS with the SIM approach. SMGs content could be quantified based on ion characterization resulting from a mass detector (m z^{-1}). This method was claimed to have higher sensitivity than other methods and is able to ignore interference components. However, this method has been tested with biodiesels from used cooking oil and waste animal fats only. Till now, there has been no research that quantified SMGs in palm oil biodiesel using the GC-MS method.

Considering the above studies, the purpose of this research is to quantify SMGs in palm oil biodiesel, which is widely used as feedstock in Indonesia. Unlike the previous method, this research quantified SMG using GC-MS with representative quantification ions of biodiesel from palm oil in order to arrive at a method that could be recommended for adoption in the Indonesia biodiesel quality standard (SNI).

MATERIALS AND METHODS

Chemicals and Materials

Certified reference materials (CRM) for MGs consisting of monomiristin (1-C14:0), monopalmitin (1-C16:0), monostearatin (1-C18:0), mononononadecanoin (1-C19:0) and monoarachidin (1-C20:0), plus n-methyl-n-(trimethylsilyl)-trifluoroacetamide (MSTFA) as silylation agent, pyridine and n-hexane as solvent, were purchased from Merck. Samples of crude palm oil (CPO), olein, refined bleached deodorized palm oil (RBDPO) and stearin as biodiesel raw materials and 2 palm oil biodiesels were supplied as courtesy by the Indonesian association of biofuel producers (asosiasi produsen biofuel Indonesia APROBI).

Instrumentation

GC-MS analysis was performed on a Thermo Scientific™ ISQ™ LT single quadrupole GC-MS equipped with a TG-5MS column (30 mx0.25 mmx0.25 µm and AI AS^{-1} 1310 series autosampler. All data were analyzed by chromeleon as a computer program from thermo scientific.

GC-MS Conditions

The GC-MS conditions mentioned in Table 3 were chosen referring to a previous publication by Yang *et al*. (2011).

Quantitation Ions: Full Scanning Mode

All CRM SMGs (1-C14:0, 1-C16:0, 1-C18:0, 1-C19:0, and 1-C20:0) were prepared in concentration 1 mg mL^{-1} as stock solutions. 500 µl stock solution was put into a 20 mL volumetric flask and diluted with pyridine. 250 µl diluted solution was put into a GC vial, and heated in a

Table 3 Instrumental conditions for GC-MS

Parameter	Condition
Column	30 m x 0.25 mm x 0.25 µm
Pressure	52.2 KPa
Gas type	Helium
Oven	
Initial temperature	50 °C
Initial time	1 min
Rate	15 °C min^{-1} to 275 °C hold for 10 min
Injection	
Volume	1 µL
Mode	Split
Split ratio	10:1
Split flow	9.9 mL min^{-1}
Temperature	250 °C
MS parameter	
Acquisition mode	SIM
SIM parameters	Group 1 ions: 343, 371, 399, 413, 427
Solvent delay	4 min
Quadrupole temperature	150 °C
Source temperature	230 °C

water bath at 120 °C. The residue was silylated with 100 µl MSTFA for 1 hour, and heated again in a water bath at 140 °C. 0.5 mL hexane was added before it was analyzed using GC-MS.

Linearity

All CRM SMGs stock solutions were serially diluted in 6 different concentrations of 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mg mL^{-1}. Derivatization was carried out by adding 10 µL MSTFA into each GC vial, vortexing for 1-2 minutes, and then settling them at room temperature for 30 minutes. Those serial samples were analyzed with the corresponding GC-MS method described in Table 1.

Limit of Detection (LOD) and Quantification (LOQ)

LOD and LOQ were determined at very low concentrations (0.1, 0.05, 0.025 µg mL^{-1}) in triplicates and by measuring the signal to noise (S N^{-1}) of each SMG. These measured ratios allowed the calculation of the corresponding concentrations for LOD at a signal-to-noise ratio of three (S N^{-1}=3), and LOQ at a signal-to-noise ratio of nine (S N^{-1}=9).

Preparation of Palm Oil Biodiesel

250 mL of raw material was put into a reactor equipped with a magnetic stirrer. The raw material was heated to 40 °C and the mixer was turned on. 2.5 gr KOH was dissolved in 125 mL methanol in a beaker glass, then the solution was added into the reactor and heated further up to 65 °C and kept at this temperature for 1 hour. Then, the mixture was settled in a separating funnel, the top layer was separated and washed with 250 mL water at 65 °C and heated at 105 °C to remove excess water and other volatile components. Finally, the biodiesel was filtered using 0.8 µm filter paper.

Quantification of SMGs in Biodiesel

The biodiesel samples were analyzed by using GC-MS with internal standard (IS) 1-C19:0. The IS solution was prepared with a concentration of 50 µg mL^{-1} by transferring 500 µL of IS stock solution (with concentration 1 mg mL^{-1}) into a 10 mL volumetric flask and making it up to the mark with pyridine. A 10 mg biodiesel sample was weighed accurately and put into the GC vial. Then 500 µL IS with a concentration of 50 µg mL^{-1} and 100 µL MSTFA were added. This was mixed for 1-2 minutes and settled for 30 minutes and analyzed by using GC-MS with conditions described in Table 1. The results were corrected by considering the Correction Factors (CF) shown in Equation 1.

$$CF = \frac{RF_{IS}}{RF_X} \cdots (1)$$

RF_x, Response factor of target peak;
RF_{IS}, Response factor of the IS;
CF_x, Correction factor of target peak.
RF is related to peak area as shown in Eq 2–3 below:

$$RF_X = \frac{A_X}{C_X} \cdots (2)$$

$$RF_{IS} = \frac{A_{IS}}{C_{IS}} \cdots (3)$$

A_x, Peak area of target MAGs;
C_x, Concentration of target peak;
A_{IS}, Peak area of IS;
C_{IS}, Concentration of IS.

RESULTS AND DISCUSSION

Quantitation Ions

All SMGs stock solutions (1-C14:0, 1:C16:0, 1-C18:0, 1-C19:0, 1-C20:0) were analyzed by using GC-MS. Yang *et al*. (2011) interpreted fragment ions of MAGs for 1-C16:0 and 1-C18:0 while Hirschegger *et al*. (2013) interpreted fragment ions for 1-C19:0. The quantitation ions full scanning mode are presented in Table 4.

In general, the SMG ion fragmentation pattern consists of $(M)^+$, $(M\text{-}CH3)^+$, $[M\text{-}103)^+$ and $(RCO)^+$. The ions are formed after ionizing electrons at the same temperature. The ions become unstable and break into their ion fragment m z^{-1}. Based on the fragmentation, the fragment $(M\text{-}103)^+$ was selected as a quantitative ion for quantification of SMGs in biodiesel because it has a high abundance compared to the others. For example, monopalmitin when released to the detector will lose 1 electron and the parent ion will have a positive charge $(M)^+$. Silylated monopalmitin has a fragmentation with molecular weight of 474 g mol^{-1}. The biggest fragment was the ion m z^{-1} 371 where the parent ion released 1 methoxytrimethylsilane-fragment $(M\text{-}103)^+$. Hirschegger (2013) also found ions at m z^{-1} 459, 371 and 239 for 1-monopalmitin and found m z^{-1} 371 as the highest abundance.

LOD and LOQ

Another important parameter of instrumental analysis with GC-MS was the limit of detection (LOD) and the limit of quantification (LOQ). The LOD and LOQ, both relevant validation parameters, were determined by analyzing the saturated SMGs with this method at very low concentrations in triplicates and measuring the signal-to-noise-ratio (S N^{-1}) of each SMG. The LOD and LOQ are very important to show the limitation of an instrumental analysis. The LOD and LOQ of representative chromatogram of saturated MAGs (1-C16:0) on LOD and LOQ determination in 3 different concentrations is shown in Figure 1.

The LOD and LOQ of all SMGs are listed in Table 5. Monopalmitin (1-C16:0) has the lowest LOD and LOQ due to the abundance of monopalmitin compounds with fragmentation of the main ion (M-103) m z^{-1} which is very large compared to the other SMGs at every measurement.

Linearity

The sensitivity of a method is shown by its linearity. Figure 2 plots a concentration series of SMGs against peak area to get linear regression lines. The coefficients of determination R^2 of all target MGs are >0.99, which is acceptable for this application.

Quantification of SMG in Biodiesel

The determination of the correction factor for quantification of SMGs in biodiesel was conducted by using 1-C19:0 as the internal standard. As a calculation reference, the other SMGs will be corrected by using the reference. The result of the correction factor calculations based on the average data in triplicates are listed in Table 6.

The biodiesel samples that were analyzed consisted of 6 biodiesels derived from CPO, olein, PFAD, stearin and RBDPO. Two palm oil biodiesels supplied by the APROBI were also analyzed. The representative gas chromatogram of biodiesel from the CPO sample is shown in

Table 4 Quantitation ions and characteristic mass spectra of MAGs

CRM	$(M)^+$ m z^{-1}	$(M\text{-}CH_3)^+$ m z^{-1}	$(M\text{-}103)^+$ m z^{-1}	$(RCO)^+$ m z^{-1}
Monomyristin (1-C14:0)	446	431	343	211
Monopalmitin (1-C16:0)	474	459	371	239
Monostearin (1-C18:0)	502	487	399	267
Mononondecanoin(1-19:0)	516	501	413	281
Monoarachidin (1-C20:0)	530	515	427	295

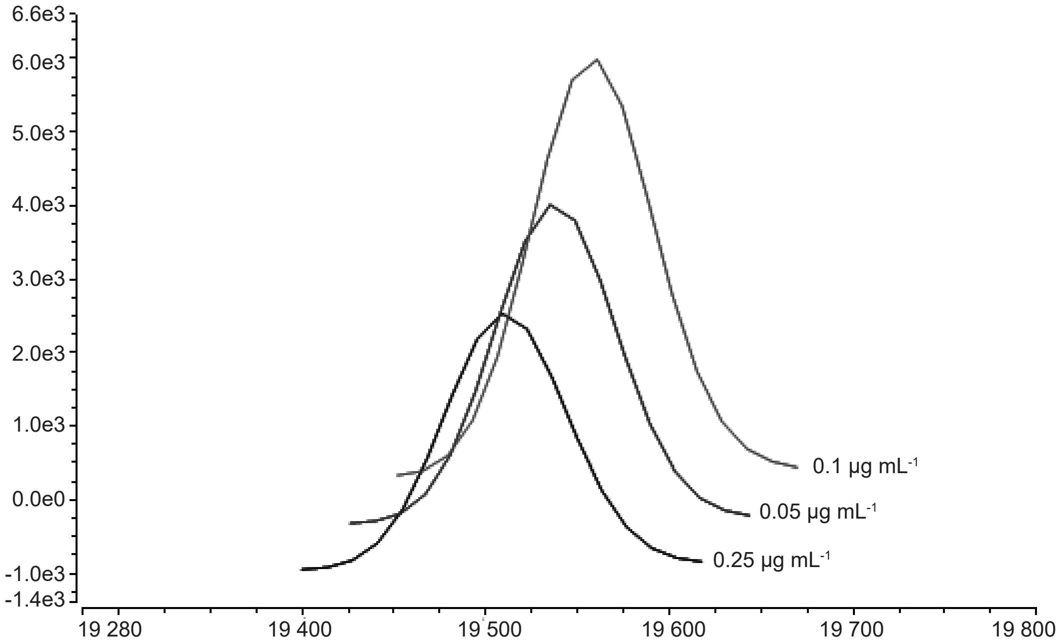

Figure 1 Representative of chromatogram for limit of detection and limit of quantification determination (monopalmitin: 0.1, 0.05, 0.025 µg mL^{-1}).

Table 5 limit of detection and limit of quantification as ppb by weight

Parameter	LOD (ppb)	LOQ (ppb)
1-C14:0	19.79	59.37
1-C16:0	1.48	4.43
1-C18:0	23.97	71.92
1-C20:0	18.06	54.18

Figure 3. As expected, the CPO feedstock contained mostly monopalmitin (1-C16:0). The results of quantification of saturated SMGs in palm oil biodiesel are listed in Table 7. Stearin biodiesel contained the highest saturated SMGs (0.333%-mass).Because the highest fatty acid

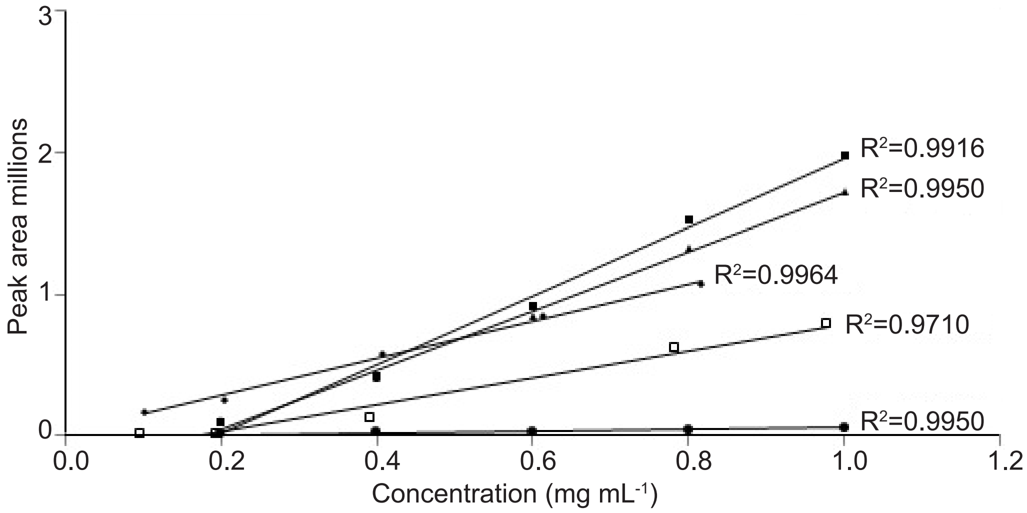

Figure 2 Standard curve of the saturated monoglycerides model.
▴1-C18:0, ⬩1-C14:0, ▪1-C16:0, ▫1-C19:0, ● 1-C20:0.

Table 6 Correction factor of the saturated monoglycerides model

SMG	Concentration (μg mL^{-1})	Area	RFx	CFx
1-C14:0	0.051	189 387	3 713 467	0.47
1-C16:0	0.050	141 768	2 835 358	0.62
1-C18:0	0.050	87 206	1 744 121	1.01
1-C19:0	0.049	86 183	1 758 833	1.00
1-C20:0	0.050	38 190	762 893	2.31

Table 7 SMG content in palm oil based biodiesel

Biodiesel	GC-MS (%-mass)
CPO	0.302
Olein	0.189
RBDPO	0.208
Stearin	0.333
PT. A	0.136
PT. B	0.151

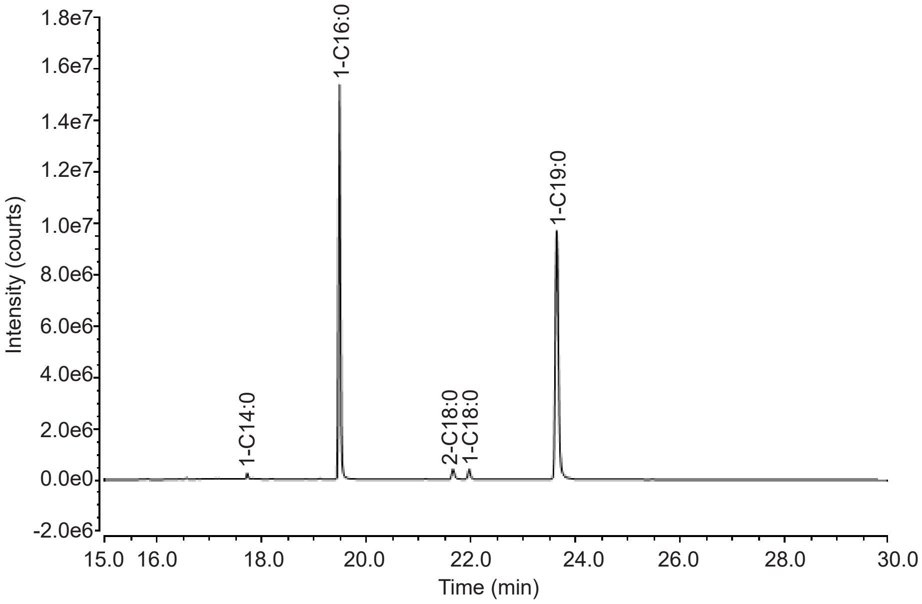

Figure 3 Representative chromatogram GC-MS of crude palm oil.

component in stearin is palmitic acid (Hasibuan 2012), this is a quite natural result. As a consequence, the use of stearin as feedstock is not recommended.

CONCLUSIONS

In this research, quantitative analysis of SMG in palm oil biodiesel oil was conducted using the GC-MS method. The results showed that the quantitation of 1-C14 ions for 1-C16:0, 1-C18:0, 1-C19:0 and 1-C20:0 were 357, 371, 399, 413 and 427 respectively. The LODs of this method were 1.48 ppb-23.97 ppb and LOQs were 4.43 ppb-71.92 ppb. This method showed a highly correlative response indicated by linearity with r=0.996-0.998. The highest SMG content in palm oil based biodiesel is monopalmitin (1-C16:0). Among palm based biodiesels, those derived from stearin contained the highest SMG (0.333%-mass).

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