A Simple Capillary Column GC Method for Analysis of Palm Oil-Based Polyol Esters

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ABSTRACT: A reliable, simultaneous analysis of palm oil polyol esters using capillary column GC is described. The polyol esters were esterified from palm oil methyl esters (POME) and palm kernel oil methyl esters (PKOME) with trimethylolpropane [2-ethyl-2-(hydroxymethyl)-1,3-propanediol; TMP] to produce the biodegradable base oil for lubricant production. Analysis was performed using a high-temperature capillary column, SGE HT5 operated at a temperature gradient of 6°C/min starting from 80 to 340°C. Before injection, the sample was derivatized with *N,O*-bis(trimethylsilyl)trifluoroacetamide in ethyl acetate at 40°C for at least 10 min. This procedure provides a complete separation of reaction products: TMP, methyl esters, monoesters (ME), diesters (DE), and triesters (TE). As expected, the ME from palm kernel oil was resolved into five major peaks, DE into seven peaks, and TE into 10 peaks. Since no standard was available for this sample, the identities of the peaks were established by a secondary standard that was derived from pure methyl esters. This GC method has made possible the simultaneous determination of reaction product compositions in order to assess the extent of reaction.

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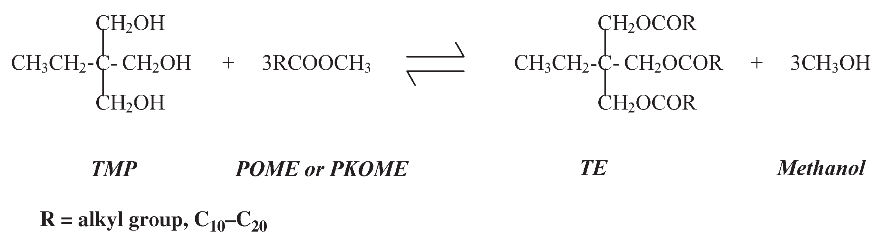
KEY WORDS: Biodegradable lubricant, gas chromatography, palm oil, palm oil methyl esters, polyol esters, transesterification.

There is a growing interest in the development of biodegradable lubricants from vegetable oils (1–4). However, characteristics inherent to biodegradable lubricants, such as inadequate oxidative stability, poor corrosion protection, and poor low-temperature performance, are major impediments to their potential use as lubricants. Of these, oxidative stability is the major practical disadvantage of using vegetable oils. Uosukainen *et al.* (2) and Wu *et al.* (3) have proposed chemical modifications, namely, transesterification and epoxidation, to improve the oxidative stability of rapeseed oil-based lubricant. Genetic modification and selective hydrogenation also are used to improve the oxidative stability (1).

Polyol esters (PE) are products of transesterification of FA or FA esters with a polyol such as trimethylolpropane (TMP). PE have been developed as base oil for various types of lubricating oil and prepared in various ways (2–9). In this work, PE were synthesized from palm oil-based and palm kernel oil-based methyl esters (POME and PKOME, respectively) with TMP (Scheme 1). Since TMP has three hydroxyl groups, three groups of esters of TMP could be formed, namely, monoesters (ME), diesters (DE), and triesters (TE). The lubricating properties of the TMP esters depend primarily on the structures of these esters. Base oil with high TE content has superior temperature behavior compared to the oil with more ME and DE. The remaining hydroxyl groups present in these partial esters have caused the thermal stability of the oil to fall (7).

For the purpose of process development and quality control, a reliable and reproducible method for the analysis of these polyol esters and intermediate products is needed. The most commonly used method is high-performance gel permeation chromatography with a refractive index (RI) detector (3,9). In this method, the components are eluted on the basis of their molar masses. However, an RI detector is not designed for gradient elution; hence, the separation of different acyl groups is not possible. Moh *et al.* (10) recommended the use of ELSD to improve the separation of sucrose esters. Other studies have analyzed higher esters, but no single method is yet successful in the simultaneous separation of polyol, methyl esters, and different groups of polyol esters formed from a reaction on a single run using GC. In this paper, we describe a GC method for the simultaneous analysis of reaction products from the transesterification of POME and PKOME and TMP to polyol esters. For comparison, the



SCHEME 1

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samples were also analyzed using HPLC coupled with ELSD and TLC.

EXPERIMENTAL PROCEDURES

Materials. POME and PKOME were obtained from Carotech Sdn. Bhd. (Ipoh, Perak, Malaysia) and Cognis Oleochemicals (Teluk Panglima Garang, Selangor, Malaysia) Sdn. Bhd., respectively. TMP [2-ethyl-2-(hydroxymethyl)-1,3-propanediol] was purchased from Merck-Schuchardt (Hohenbrunn, Germany); sodium methoxide, THF, sodium acetate, and reference standards were purchased from Sigma-Aldrich Chemicals Co. (Steinheim, Germany). *N,O*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) was purchased from Fluka Chemie AG (Buchs, Switzerland).

Standard solutions. Approximately 0.03 ± 0.005 g of sample was weighed exactly into a 5-mL vial and diluted with 1.0 mL of ethyl acetate. The sample was swirled for a few minutes to dissolve the mixture. BSTFA (0.5 mL) was then added to the mixture and swirled. The vial was then transferred to a water bath held at 40ºC for 10 min. This procedure allowed the silylation of the sample to take place. The sample was then removed from the water bath and transferred to a 2-mL auto sampler vial for injection into the GC system.

Test samples. Samples were taken from the experiments on the transesterification of POME and TMP to polyol esters.

Validation. Since no standard for TMP-based ME, DE, and TE was available commercially, the solutions were synthesized from pure methyl esters, namely, methyl laurate, methyl palmitate, and methyl stearate. These esters were reacted with TMP using sodium methoxide as a catalyst. The methyl ester-to-TMP molar ratio and catalyst weight percentage were fixed at 3.9:1 and 0.9%, respectively, to ensure optimal conversion of polyol esters (2). The reactions were carried out at 130ºC and 1.0 kPa for 5 h. The reaction products were neutralized to remove the catalyst and then distilled under vacuum to take away the excess methyl esters.

TLC. TLC was performed according to a procedure modified from Linko *et al.* (4). Separation was achieved on Kieselgel $60F_{254}$ TLC plates (Merck, Darmstadt, Germany) with *n*-heptane/ethyl acetate as solvent. Two different solvent ratios were required to resolve the spots for ME/DE and POME/TE. Ratios of 83:17 and 95:5 (*n*-heptane/ethyl acetate) were used for the separation of ME/DE and POME/TE, respectively. The thin-layer plate was developed, dried in a fume hood, and sprayed with a mixture of 0.05 wt% 2′,7′-dichlorofluorescein in absolute ethanol or placed in an iodine chamber until the spots appeared.

HPLC. The HPLC system was equipped with a binary gradient pump, an automatic, three-line degasser, a column oven, and an ELSD. Data were recorded and analyzed using Windows-based Intuitive Software for Chromatography (Borwin, version 1.21; JBMS Développements, Le Fontanil, France). A packed Inertsil ODS 3 column (4.6 × 250 mm; GL Science Inc., Rockford, IL) was used for separation. The column was maintained at 30ºC, and the mobile phase used was a mixture of 60:40 acetonitrile (ACN) and dichloromethane (DCM) at a flow rate of 0.8 mL/min. The ELSD detector was set at 60ºC and run under 2.3 bar air pressure. The sample solution (approximately 0.02 g in 10 mL THF) was passed through a 0.45-µm disc filter and transferred to an auto sampler vial. An aliquot of about 20 µL was injected into the HPLC system.

GC. The separation on the GC system was performed using the capillary column SGE HT5, 12 m × 0.53 mm, i.d. 0.15 µm (SGE, Melbourne, Australia). The oven temperature was set initially at 80ºC, held for 3 min, then increased at 6ºC/min to 340ºC and held for another 6 min. The injector and detector temperatures were at 300 and 360ºC, respectively. Hydrogen was used as the carrier gas at a flow rate of 26.7 mL/min. The split ratio was set at 1:1, and 1.0 µL of sample was injected into the GC system

RESULTS AND DISCUSSION

FA compositions of POME and PKOME, as shown in Table 1, were determined according to the standard methods for oils and fats analysis (11). Figure 1A shows the TLC separation of ME and DE spots, whereas Figure 1B illustrates the separation of TE and POME spots. The spots closer to the origin in Figure 1B represent the more polar compounds, DE and ME, which could not be resolved. By changing the *n*-hep-

TABLE 1
Average FA Compositions of Palm Oil-Based Methyl Esters (POME) and Palm Kernel-Based Methyl Esters (PKOME)

FA	POME (%)	PKOME (%)
10:0	—	0.1
12:0	0.9	48.9
14:0	1.5	18.8
16:0	41.5	10.2
16:1	0.3	—
17:0	0.1	—
18:0	2.7	2.7
18:1	40.6	17.0
18:2	11.9	2.2
18:3	0.3	—
Others	0.2	—

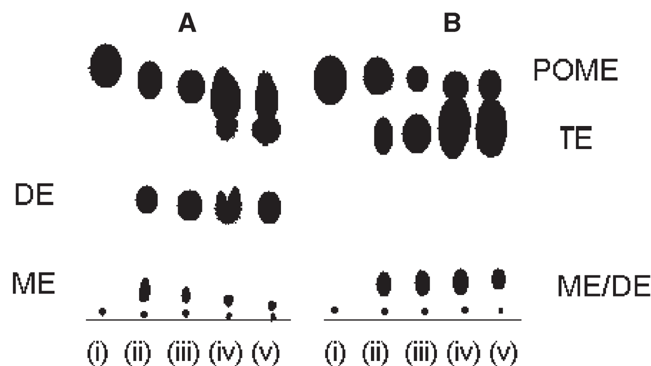


FIG. 1. TLC plates showing the elution of partial polyol esters at two different solvent ratios. (A) *n*-Heptane/ethyl acetate, 83:17; (B) *n*-heptane/ethyl acetate, 95:5. ME, monoester; DE, diester; TE, triester; POME, palm oil-based methyl ester. Sampling times: (i) Initial, (ii) 15 min, (iii) 30 min, (iv) 45 min, (v) 60 min.

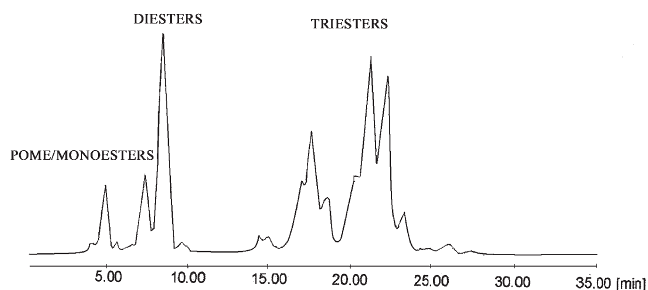
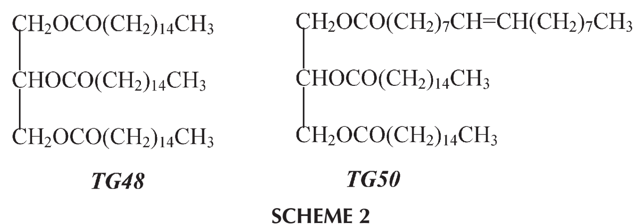


FIG. 2. HPLC chromatogram of palm oil-based polyol esters derived from the reaction between POME and trimethylolpropane [TMP: 2-ethyl-2-(hydroxymethyl)-1,3-propanediol]. Conditions: molar ratio of TMP to POME, 1:3.9; sodium methoxide, 0.9%; 140°C; 0.3 mbar; 5 h. For abbreviation see Figure 1.

tane/ethyl acetate ratio from 95:5 to 83:17, thus increasing the polarity of the mobile phase, the spots were successfully separated into ME and DE as shown in Figure 1A. This separation is crucial because the progress of reaction was monitored semiquantitatively by the successive disappearance of ME and DE spots.

The substrates in this transesterification reaction consist of lipophilic (PKOME) and hydrophilic (TMP) substances. The wide range of their polarities (lipophilic and hydrophilic) and their insolubility in many solvents have caused many researchers to exclude the use of HPLC. Most of them resort to other simpler methods, such as TLC (6) and sometimes include FID (TLC–FID), to capture the separation of components more effectively (7). Nevertheless, we made an attempt to use HPLC in our work and use a compatible solvent (THF) to dissolve the substrates and reaction products. This effort was partially successful, as shown in Figure 2 for palm oil-based reaction products. There is a distinct separation between DE and TE but not between methyl esters and ME of TMP. The latter overlap; hence, it is impossible to assess the disappearance of methyl esters and formation of ME during the progress of the transesterification. Peak overlapping was also reported in the transesterification of rapeseed oil with excess 2-ethyl-1-hexanol (8). The substrate peak (2-ethyl-1-hexanol) overlapped the solvent peak, and hence the residual amount could not be determined. Elfman-Börjesson and Härröd (12) used an HPLC method on a diol column and experienced similar problems dealing with components having similar polarity. The identification of the HPLC peaks in this work was carried out by quantitative TLC; individual spots were developed, scraped off the plates, dissolved in diethyl ether, and recovered by vaporizing the solvent in a N_2 stream. The experimental procedure was similar to that followed for qualitative TLC. The sample was then injected into HPLC for identification purposes.

PE are molecules of high M.W. (above 900 for TE) and low volatility, so HPLC is prefered to GC. However, in our work, we discovered that methyl ester peaks on the HPLC chromatogram disappeared together with the ME peaks as the reaction progressed. This seemed unusual because the methyl esters were in excess, should be present in the final mixture, and should appear on the chromatogram. Hence, HPLC failed



SCHEME 2

to provide good separation for these polyol esters. Consequently, the GC method was examined because it provides better separation than HPLC in most cases. GC technique for carbon analysis of oils and fats has been well established for lipid research and quality control (11). GC separates TG molecules according to the number of carbon atoms in them. Each peak in the chromatogram is referred according to its carbon number in the acyl chain of the TG molecules. For example, tripalmitin with three palmitic acid (C16) chains is referred to as TG48 and oleopalmitin with two palmitic acid chains and one oleic acid chain is TG50 (see Scheme 2). TG of the same carbon number elute together as one peak on the chromatogram regardless of the structural configuration of the TG.

In this work, the presence of components with a wide range of volatilities necessitates the use of a high-temperature column with a suitable temperature program. Figures 3A and 3B show gas chromatograms for palm kernel oil-based and palm oil-based polyol esters, respectively. The analysis was performed using a high-temperature capillary column, SGE HT5, operated at a temperature gradient of 6°C/min from 80 to 340°C. As anticipated, more than 10 major peaks were detected for palm kernel-based TE due to its wider range of FA compositions. This separation was unattainable earlier. For instance, Gupta *et al.* (13) successfully separated sucrose ME and DE using GC but not higher esters such as TE and tetra esters. In their work, the use of capillary column GC resulted in a large number of peaks that could be neither identified nor used for quantitative analysis.

The identification of the esters groups was carried out by using samples obtained from qualitative TLC as described earlier. All component peaks, namely, of methyl esters, ME, DE, and TE, were well separated. Furthermore, since the sample was derivatized, the peaks were resolved in a single analysis, not only according to their groups but also, and more importantly, according to carbon numbers as shown in Figures 3A and 3B in a single analysis. Through silylation, all of the OH groups are replaced with the methylsilyl ($–SiMe_3$) group, which improves the separation and resolution of polar compounds such as ME and DE. The separation between components of similar carbon numbers such as DE36 of diolein (OO) and TE36 of trilaurin (LLL) was also made possible by silylation. OO represents the DE of oleic acid (18:1), and LLL the TE of lauric acid (12:0). Earlier, separate analysis was usually necessary to analyze partial components in polyol esters. For instance, Hayes and Kleiman (14) had to use three different GC procedures to analyze FA, partial glycerides, and TG in the hydrolysis of lesquerella oil with lipase.

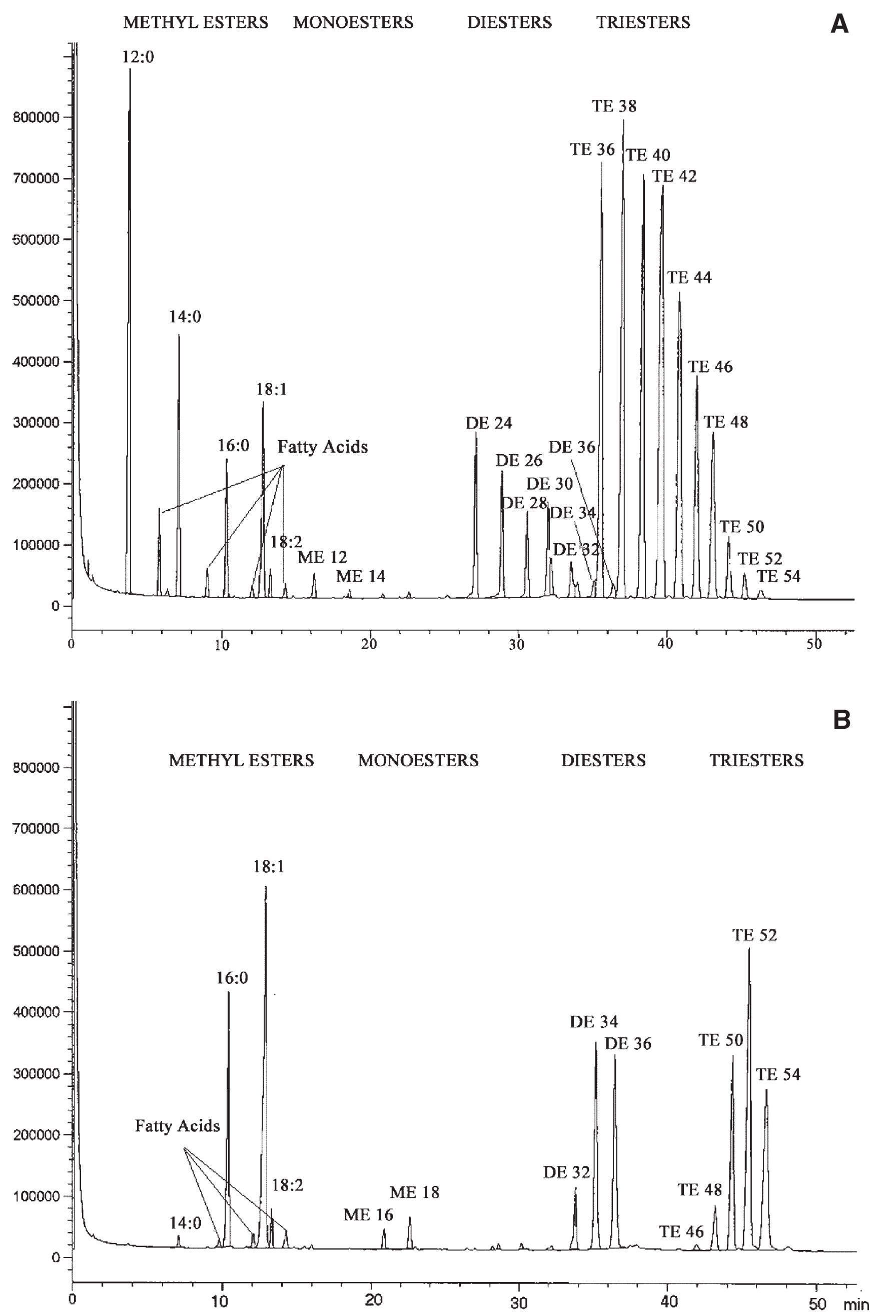
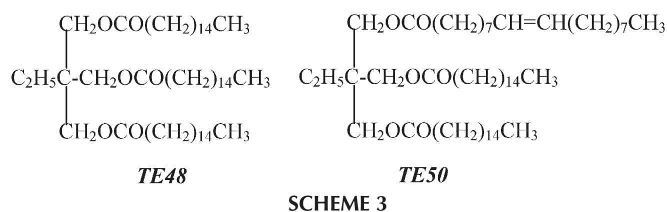


FIG. 3. Gas chromatograms of palm oil-based polyol esters derived from reaction between POME and TMP: (A) palm kernel oil methyl esters (PKOME); (B) POME. Conditions: molar ratio of TMP and POME, 1:3.9; sodium methoxide, 0.9%; 120°C; 0.1 mbar; 5 h. For abbreviations see Figures 1 and 2.

The peaks in the gas chromatogram were identified and validated by injecting known compounds derived from the reaction between methyl laurate, methyl palmitate, and methyl stearate with TMP. It was not necessary to separate and purify the individual reaction products because the peaks of all the intermediates appeared distinctively on the chromatogram. These peaks are identified and labeled according to the carbon number of the alkyl groups attached to the TMP skeleton following the same terminology used in fats and oils analysis (see Scheme 3). For instance, a reaction of pure methyl palmitate with TMP will produce polyol esters identified as monoester, ME16, if only one of its –OH groups is esterified; diester, DE32, if two –OH groups are esterified; and triester, TE48, if all –OH are esterified. The retention times of each of these partial esters is given in Table 2.

By comparing the GC peaks of primary standards consisting of TG, diglycerides (DG), and monoglycerides (MG), namely, TG48, TG54, DG36, DG32, and MG16, it was discovered that their peaks coincided with polyol ester peaks at two extra carbon number locations. For instance, the TE48



SCHEME 3

peak appears at the same retention time as TG46, and TE54 coincides with the TG56 peak. This means that standards for partial glycerides could be used in cases where polyol esters standards are not available. However, a thorough calibration procedure must be followed prior to injection. In this work, the choice of solvent is also critical because a certain type of polar solvent, namely, THF, reacts with a silylation agent such as BSTFA. Consequently, many ghost peaks would appear on the chromatogram. The GC chromatogram in Figure 3 illustrates the identification of peaks for polyol esters derived from POME and PKOME.

GC analysis could also be used to monitor the progress of the transesterification reaction. Figures 4A–D illustrate the appearance and disappearance of certain peaks during the course of reaction. Since there are three –OH groups in TMP, three progressive reactions occurred in the process with the intermediate formation of ME, DE, and TE of TMP, respectively. It appears from the chromatogram that the reaction was very rapid due to the instantaneous formation of ME and DE after the addition of catalyst (initial state). The formation of TE-peaks occurred only after the formation of the DE. Consequently, the result has indeed confirmed the mechanism of the reaction to be stepwise reactions. After 30 min of reaction, the TMP peaks disappeared, and in 45 min, only trace amounts of ME were visible. The reaction was considered to be complete when the areas of the TE peaks remained constant.

The GC procedure described above has several advantages over previous GC methods. Most of the reported cases were unable to separate the high M.W. esters such as TE and tetra esters, and only the ME and DE were identified (13–15). Furthermore, separate analysis was usually necessary to analyze other components in polyol esters (16). In contrast, our method allows simultaneous detection of all compounds involved in a reaction, namely, ME, DE, TE, methyl esters, and TMP. It also offers a reliable way of monitoring the progress of a reaction.

TABLE 2
Average Retention Times for Components Obtained from Reaction Between Methyl Palmitate and Methyl Laurate with Trimethylolpropane[a]

Components	Identification	Retention time (min)[b]
Trimethylolpropane	TMP	1.4
Methyl laurate	12:0	4.0
Lauric acid	FA-12:0	5.8
Methyl palmitate	16:0	10.3
Palmitic acid	FA-16:0	12.1
Monolaurate	ME12	16.2
Monopalmitate	ME16	20.8
Dilaurate	DE24	27.1
Dipalmitate	DE32	32.5
Trilaurate	TE36	35.4
Tripalmitate	TE48	43.6

[a]Component names ending with -ate are used to differentiate between TMP esters and glycerol esters (mono-, di-, and triglycerides).
[b]SD ± 0.1.

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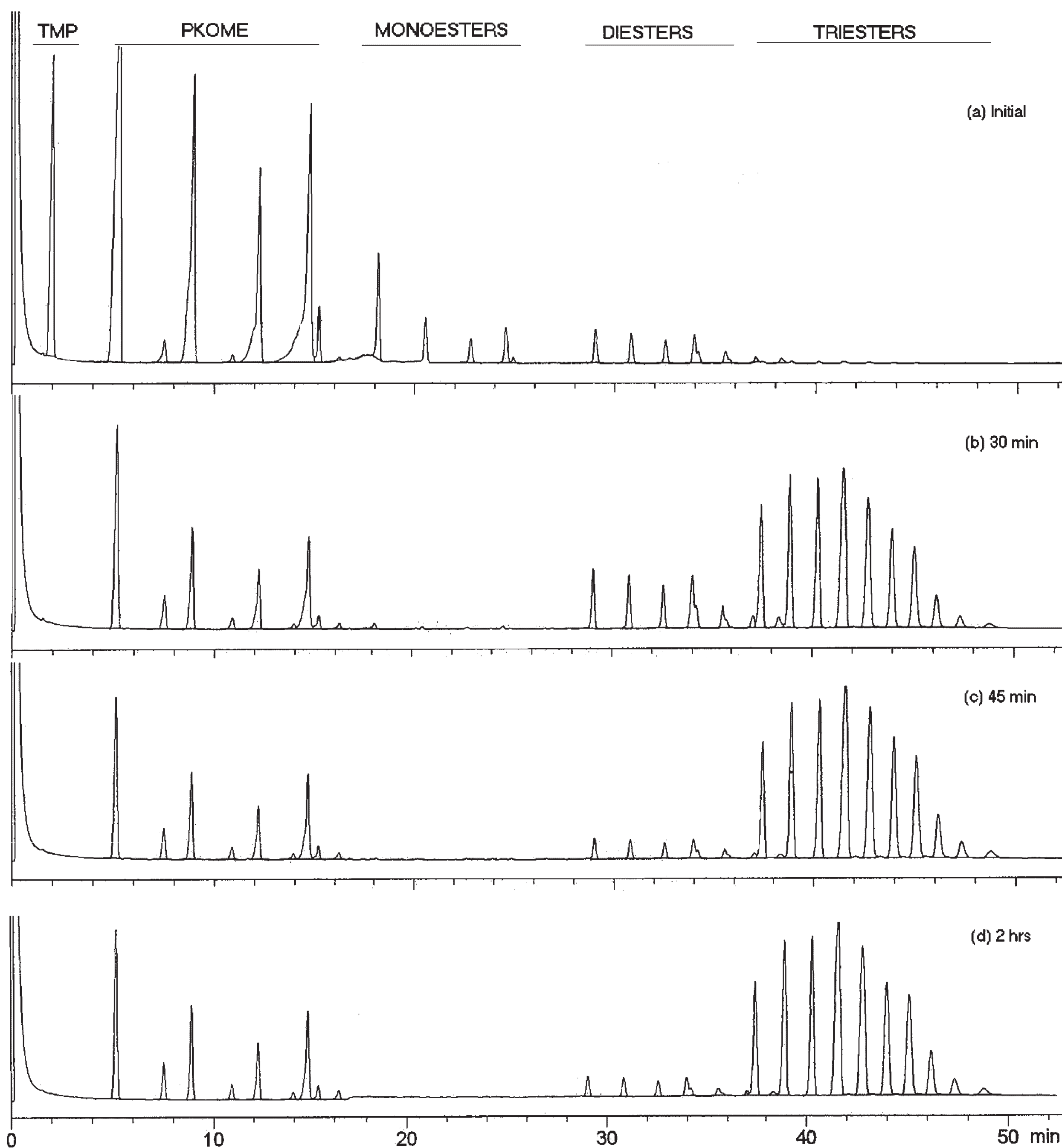

FIG. 4. GC separation showing the formation of intermediates as the reaction between PKOME and TMP progresses for 2 h. Conditions: molar ratio of TMP and PKOME, 1:3.9; sodium methoxide, 0.9%; 130°C; 10 mbar; 5 h. For abbreviations see Figures 2 and 3.

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