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Short communication

Identification of monoacylglycerol regio-isomers by gas chromatography–mass spectrometry

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ABSTRACT

Monoacylglycerols (MAGs) are lipids found in trace amounts in plants and animal tissues. While they are widely used in various industrial applications, accurate determination of the regio-specific distribution is hindered by the lack of stable, commercially available standards. Indeed, unsaturated β-MAG (or *Sn*-2 MAG) readily undergoes isomerization into α-MAG (acyl chain is attached to the *Sn*-1 or the *Sn*-3 position). In the present study, we describe structural elucidation of α- and β-regio-isomers of monopalmitoylglycerol (MAG C16:0) as model compounds in their silylated forms using gas chromatography–mass spectrometry (GC–MS) with electronic impact (EI) ionization. MS fragmentation of α-MAG C16:0 is characterized by the loss of methylene(trimethylsilyl)oxonium (103 amu) and the consecutive loss of acyl chain yielding a fragment ion at *m*/*z* 205. The fragmentation pattern of β-MAG C16:0 shows a series of diagnostic fragments at *m*/*z* 218, 203, 191 and 103 that are not formed from the α-isomer and hereby enable reliable distinction of these regio-isomers. Possible fragmentation scenarios are postulated to explain the formation of these marker ions, which were also applied to characterize the regio-isomer composition of a complex mixture of MAG sample containing n-3 long-chain polyunsaturated fatty acids.

1. Introduction

Monoacylglycerols (MAGs) are found in trace amounts in plant lipids, animal tissues and are widely used in various industrial applications due to their detergent properties. Two types of regio-isomers (α and β) can be found depending on the position of the acyl chain on the acylglycerol backbone. α-Isomers have acyl chain linked to carbon 1 or 3 of *Sn*-glycerol, while β-isomers have the acyl chain in the *Sn*-2 position. α- and β-isomers of saturated MAG are more stable than unsaturated MAGs and readily isomerize to an equilibrium state with a strong preference for the external (α) position [1]. This process happens during synthesis, storage or sample preparation and the use of boric acid has been shown to slow down the isomerization process [1].

In animals, MAGs are formed during lipase-catalyzed hydrolysis of dietary triacylglycerols in the small intestine [2]. The production of MAG and especially β-MAG is essential for the formation of mixed micelles that are absorbed by the enterocytes [2]. *Sn*-2-arachidonylglycerol (β-MAG of arachidonic acid), a cannabinoid type 1 receptor (CB1) agonist, was also reported to be implicated in the regulation of food intake and other biological functions [3,4].

MAG compositional analysis is conventionally performed by gas chromatography (GC), liquid chromatography (LC) or thin-layer chromatography (TLC) [5]. While carefully optimized LC can be used to separate MAG regio-isomers, GC analysis is suitable to determine the total fatty acid. MAG fractions isolated by TLC are often transesterified into fatty acid methyl ester derivatives and are subsequently analyzed by GC [2,5]. This type of analysis is suitable to determine the total fatty acid profile in the MAG fraction, but does not give information about the original location of the fatty acids on the glycerol backbone. For this latter purpose, gas chromatographic separation of MAG regio-isomers has been reported in their di-butyryl ester derivatives [6]. The preparation of di-butyryl esters of MAG (DBMAG) from free MAG was performed using butyryl chloride as acylation reagent [6]. This type of derivatization has also been used to determine the regio-specific distribution of fatty acids in triacylglycerols (TAG) after Grignard reagent catalyzed partial degradation of TAG in various types of oils [7–9]. Gas chromatographic separation and flame ionization detection (GC-FID) of saturated α- and β-MAG such as α-16:0, β-16:0, α-18:0 and β-18:0 as their di-TMS derivatives was published

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in standardized AOCS method [10]. Then again, general application of this method to complex samples containing other MAG homologues with different acyl chains is limited due to the unavailability of pure standards.

The use of mass spectrometer (MS) to selectively detect MAG derivatives can potentially provide further information to GC separation if fragmentation pathways are understood. GC–MS analysis may potentially allow to resolve MAG regio-isomers and provide accurate identification of those isomers without available standards. In the present study, we describe distinction and structural characterization of MAG regio-isomers as di-TMS derivatives by electron ionization MS. The proposed fragmentation pathways can be used in a generic way to identify MAG isomers in complex samples by gas chromatography–mass spectrometry (GC–MS).

2. Materials and methods

2.1. Chemicals and reagents

Pyridine (99% purity) and N,O-bis(trimethylsilyl)trifluoroacetamide/trimethylsilylchlorosilane (BSTFA:TMCS, 99:1, v/v) were obtained from Sigma–Aldrich (Saint Louis, USA). 2-Monopalmitin (β-16:0) and 1(3)-monopalmitin (α-16:0) were obtained from Larodan (Sweden). Commercial distilled MAG (Dimodan MO) was obtained from Danisco (Copenhagen, Denmark). MAG sample containing n-3 long-chain polyunsaturated fatty acids (LC-PUFA) has been obtained from Croda Chemicals (Goole, UK). The MAG sample contained mainly MAG (95.7 g per 100 g of oil) and trace amount of diacylglycerols (DAG, 4.3 g per 100 g of oil). The levels of eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids were 22 and 14 g per 100 g of oil, respectively.

2.2. Preparation of trimethylsilyl (TMS) derivatives

MAG (1 mg) sample dissolved in pyridine (200 μL) and BSTFA:TMCS, 99:1 (200 μL) was homogenized and heated at 80 °C for 45 min. MAG di-TMS derivatives were cooled down to room temperature and diluted with hexane (5 mL).

2.3. Gas chromatography

Silylated MAG were analyzed using a 5890 HP gas chromatograph (Agilent Technologies, Palo Alto, CA) equipped with a DB-5HT capillary column (15 m × 0.25 mm i.d., film thickness 0.10 μm; J&W, Palo Alto, USA). One microliter sample was injected applying a 50:1 split ratio and subsequent flame-ionization detection (FID). Injector temperature was 345 °C and detector temperature was 380 °C. Oven temperature program was the following: starting temperature was 180 °C, increased to 350 °C at 30 °C/min and maintained at this temperature for 10 min. Carrier gas (H_2) was used at constant flow mode at 2 mL/min.

2.4. Gas chromatography–mass spectrometry (GC–MS)

Silylated MAG samples were analyzed on a 6890 Series II gas chromatograph (Agilent Technologies, Palo Alto, CA) attached to a 5973N quadrupole mass selective detector (Agilent Technologies, Palo Alto, CA) equipped with electron ionization (EI) ion source. The instrument was operated in positive ion mode using the standard 70 eV electron energy. The GC injector was operated in split mode (split ratio 25:1) at 320 °C, the GC–MS interface was maintained at 320 °C. Helium was used as carrier gas under constant flow rate of 0.7 mL/min. GC separation was performed on a RTX-65 TG capillary column (30 m × 0.25 mm i.d., film thickness 0.10 μm; Restek, Bellefonte, PA). Oven temperature program was the following: isothermal at 100 °C for 1 min, then increased to 300 °C at 5 °C/min, finally increased to 340 °C for 2 min at 20 °C/min. Electron ionization mass spectra were recorded in the m/z 100–600 range.

3. Results and discussion

The MAG composition of edible lipid samples can be characterized according to the standardized AOCS method by GC-FID [10]. This is illustrated in the case of a DIMODAN M (Fig. 1A) and a n-3 LC-PUFA MAG samples (Fig. 1B). While the DIMODAN sample contains mainly α-18:1 n-9 (88%), the n-3 LC-PUFA MAG sample is much more complex. The reason for this is the heterogeneous acyl chain distribution of the sample, which was indeed confirmed by GC-FID analysis of the fatty acid profile (results not shown). Accordingly, in this complex case the GC-FID analysis reveals the MAG classes in the sample but little information on the structure and isomerism of the individual MAG constituents.

In order to obtain this structural information, EI-MS fragmentation pattern of two commercially available saturated regio-isomers: α- and β-16:0 (monopalmitin), was investigated. α- and β-16:0 were analyzed by GC-FID (Fig. 2A) and GC–MS (Fig. 2B) as di-TMS derivatives on two different GC columns confirming that baseline resolution of β- and α-MAG can be achieved [10]. According to the analysis of these standards, β-isomer elutes before the α-isomer as previously reported in the standardized method [10] and for DBMAG derivatives [6–9].

The MS spectra of α- and β-16:0 di-TMS derivatives are provided in Fig. 3. No $[M]^+$ (molecular ions) could be observed in the positive ion EI spectra of any of the investigated isomer MAG, however both compounds yielded a peak at m/z 459 that corresponds to $[M-15]^+$. This fragment ion likely results from the molecular ion after the loss of a methyl group, a frequently observed phenomenon for silylated derivatives. While there are several other common fragment ions in the spectra of the two isomers, in this

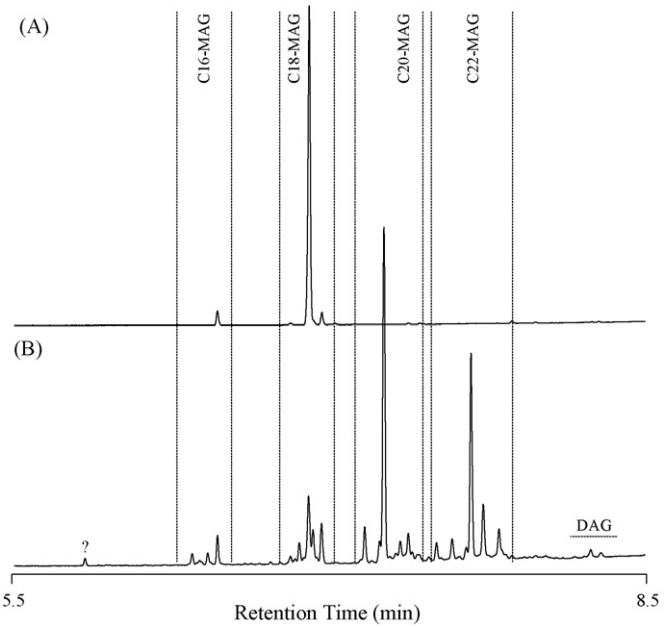

Fig. 1. Gas chromatogram of silylated (A) DIMODAN M, monounsaturated MAG from Danisco (Copenhagen, Denmark) and (B) n-3 long-chain polyunsaturated fatty acids containing MAG sample from Croda (Goole, UK). Chromatography was performed using a 15 m DB-5ht capillary column (15 m, 0.25 mm I.D., 0.1 μm film thickness, J&W, Palo Alto, CA).

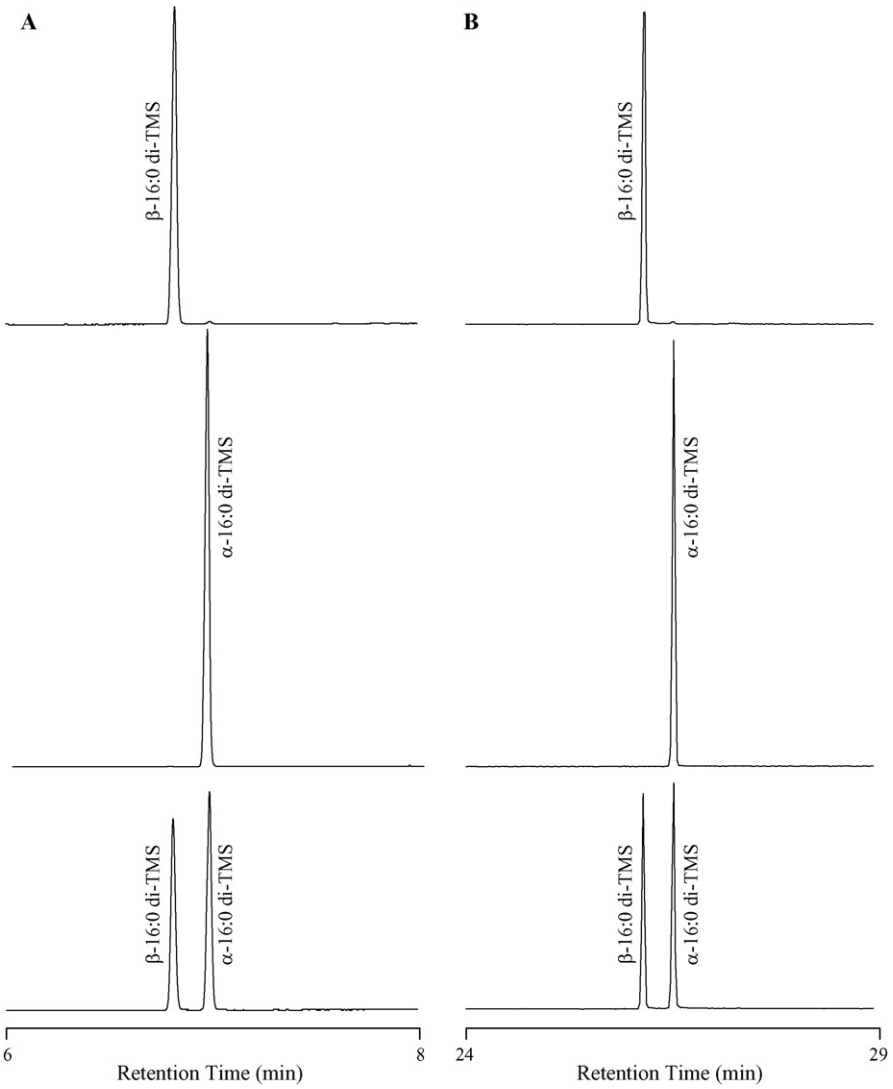

Fig. 2. Gas chromatogram of silylated Sn-2 palmitin (β-16:0 di-TMS) and Sn-1(3) monopalmitin (α-16:0 di-TMS) on (A) a non-polar DB-5ht (15 m, 0.25 mm I.D., 0.1 μm film thickness) and (B) mid-polar RTX-65 TG (30 m, 0.25 mm I.D., 0.1 μm film thickness).

manuscript we focus on those ions that enable distinction of these isomers.

A diagnostic ion is present in the spectrum of α-isomer at *m*/*z* 371 likely corresponding to the loss of a methylene(trimethylsilyl)oxonium (−103 amu) and a methyl group from the molecular ion (*m*/*z* 474, not observed), see Fig. 4. In addition, another marker ion can be observed at *m*/*z* 205 which is absent in the spectrum of the β-isomer (Fig. 3A). The postulated mechanism for the formation of this ion is shown in Fig. 4 depicting the loss of the fatty acid plus one carbon originating from the glycerol backbone via an alpha-cleavage followed by the loss of a methyl group from the trimethylsilyl part. The mass spectrum of the silylated β-16:0 (*Sn*-2 monopalmitin) exhibits an intense discriminative ion at *m*/*z* 218.1 (see Fig. 3B) that is postulated to be formed by the loss of the acyl moiety through hydrogen rearrangement, as shown in Fig. 5. This fragment ion further undergoes loss of a methyl group giving rise to a specific fragment at *m*/*z* 203.0 as shown in Fig. 5. The fragmentation of silylated α-16:0 [*Sn*-1(3) monopalmitin] (Fig. 3A) also shows a characteristic ion at *m*/*z* 191 corresponding to the silyl ether plus carboxyl moiety. This ion is proposed to be formed from the molecular ion after elimination of a C_3H_8Si residue, hydrogen rearrangement with ring condensation and alpha-cleavage of the alkane chain (see Fig. 4). Accordingly, the fragments *m*/*z* 218, 203 and 191 are characteristic for the β-isomer, while the ions *m*/*z* 371 and 205 are characteristic for the α-isomer.

Based on these observations the regio-distribution of the fatty acids esterified to the MAG was determined in a n-3 LC-PUFA sample by GC–MS after silylation. First, the chain length of the fatty acids was calculated using diagnostic fragment ions that correspond to $[M-15]^+$. In the second step, α-isomers were identified by searching for the characteristic loss of 103 amu, corresponding to methylene(trimethylsilyl)oxonium. The identification of these fragment ions revealed that most of the MAG present in this sample are *Sn*-1(3) isomers (Fig. 6). However, by plotting the ion chromatograms for the *m*/*z* 218 ions diagnostic for β-isomers, it was possible to identified trace amounts of β-16:0, β-18:0 and β-18:1 MAG derivatives in the sample (mass spectra results not shown). On the semi-quantitative basis, also integration of the observed peaks was performed and this revealed that in the n-3 LC-PUFA MAG sample, α-MAG represent 98.6% of total MAG and approximately 1.4% MAG are present in the β form. This result is in accordance with the natural isomer distribution of unsaturated MAG and confirms that these compounds indeed easily undergo isomerization [1].

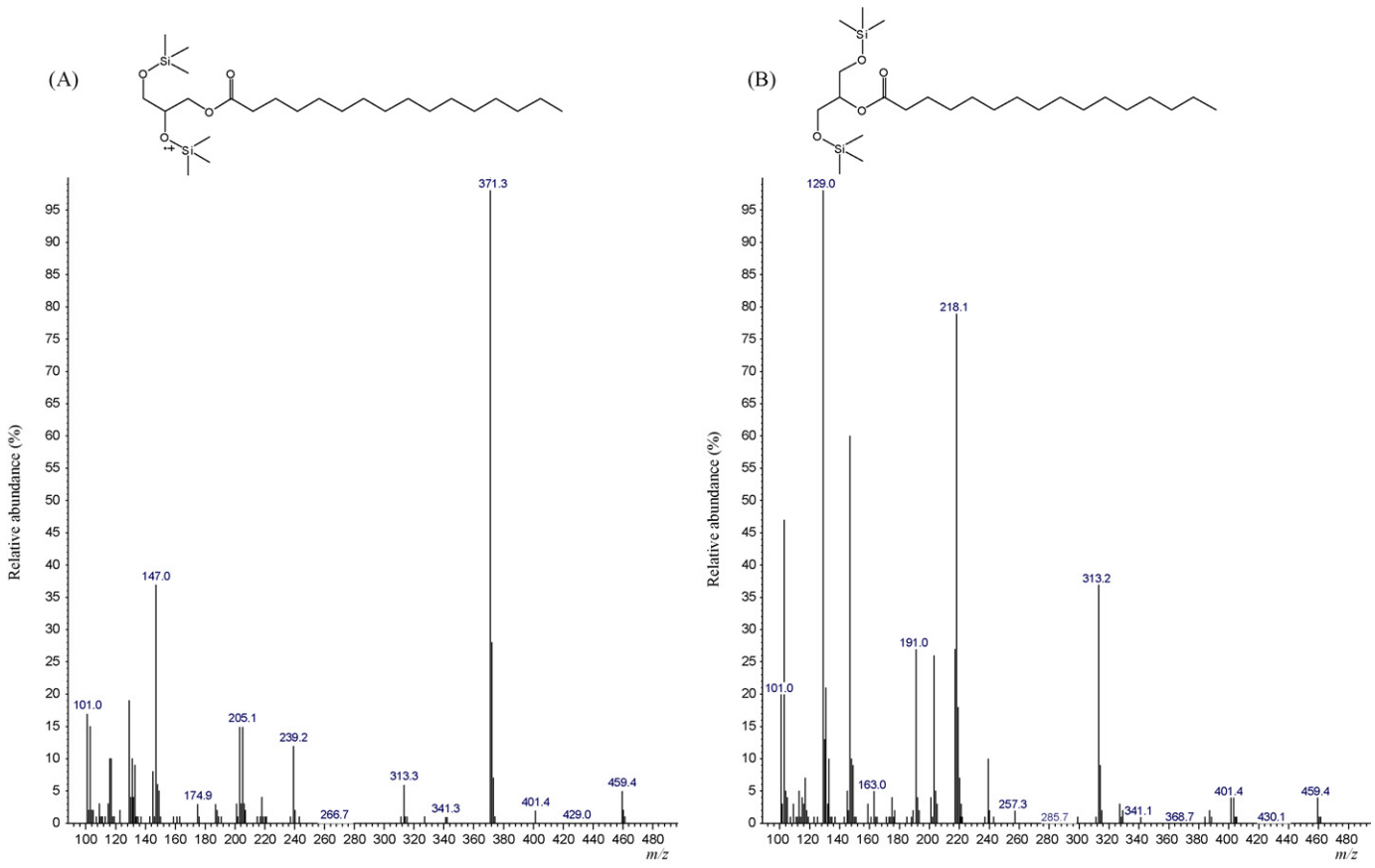

Fig. 3. EI mass spectra of silylated (A) α-16:0 and (B) β-16:0.

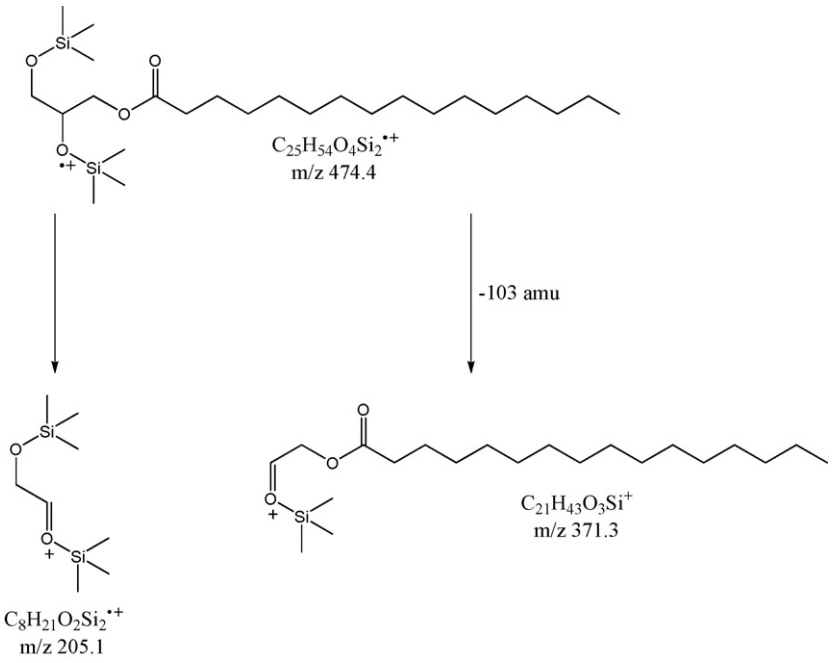

Fig. 4. EI-MS fragmentation scheme of silylated α-16:0.

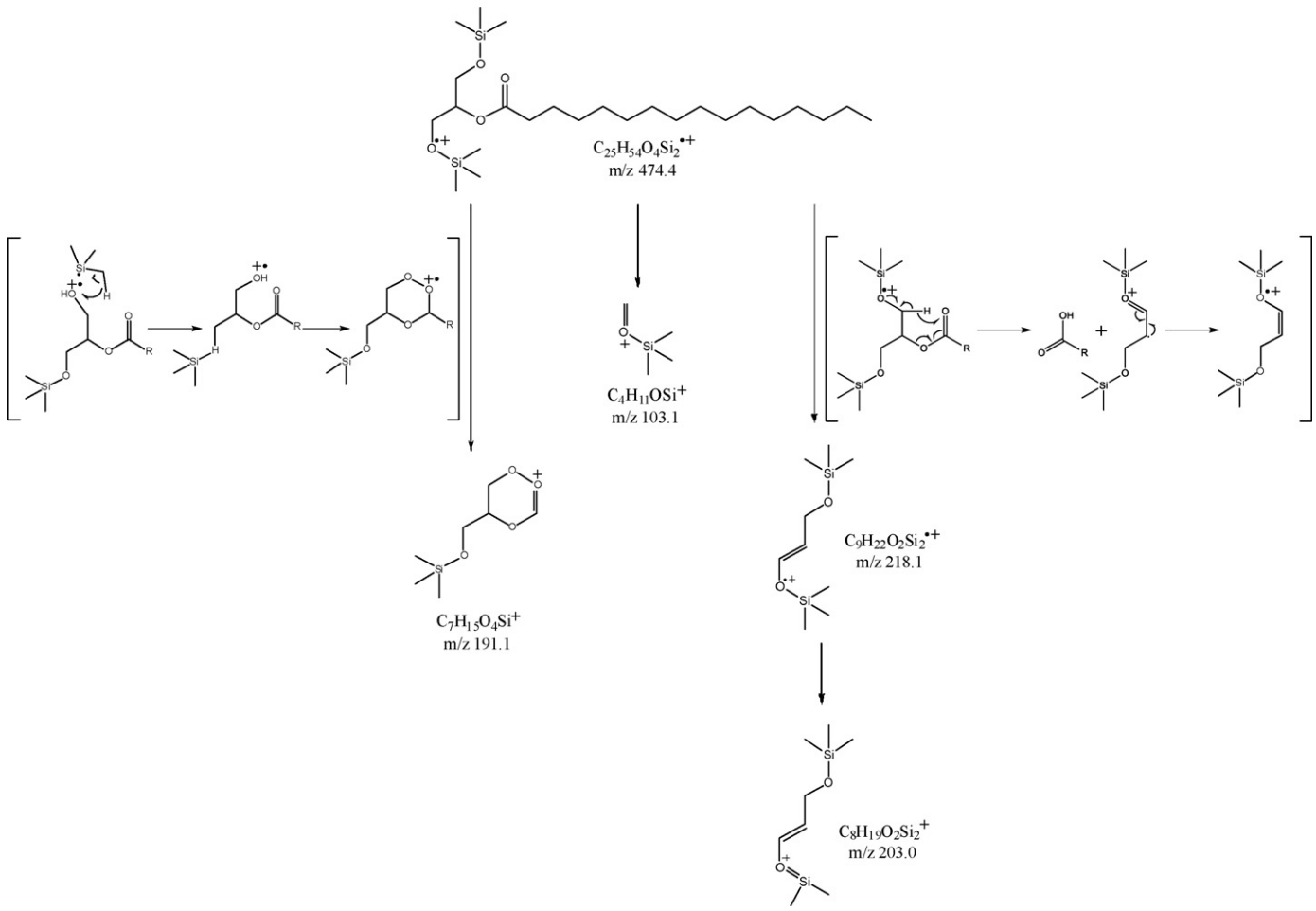

Fig. 5. EI-MS fragmentation scheme of silylated β-16:0.

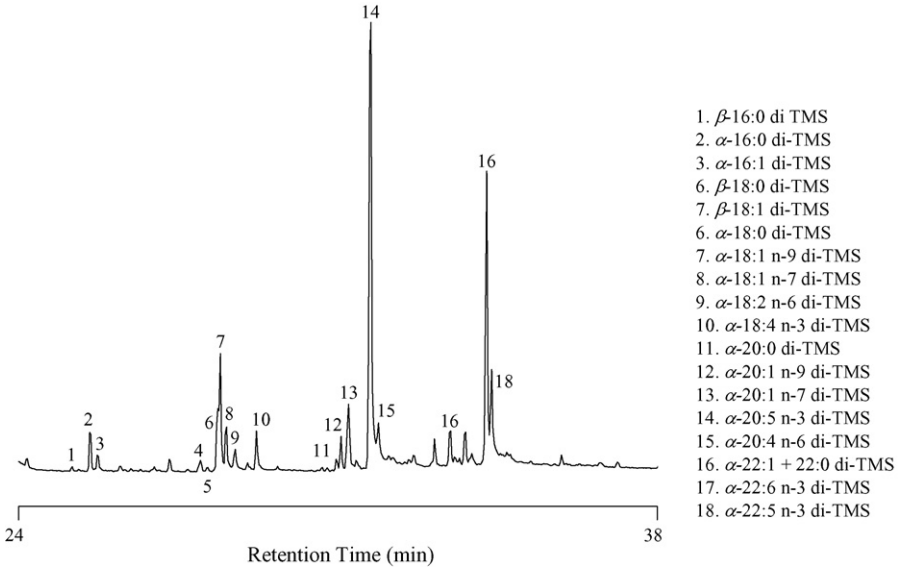

Fig. 6. GC–MS analysis (total ion current) of a silylated MAG sample (Croda, Goole, UK) containing n-3 long-chain polyunsaturated fatty acids. Identification of the regio-isomers has been performed according to diagnostic ion fragments as described in Section 3.

4. Conclusion

The main limitation in the analysis of MAG regio-isomers is that standards of unsaturated β-MAG are not available because these molecules isomerize readily into α-MAG. In this manuscript, GC–MS is proposed to distinguish between the α-and β-isomers and to enable unequivocal identification of regio-isomers without the need of pure standards. Fragmentation mechanisms are postu-

lated for the formation of the observed diagnostic ions in the cases of both α- and β-isomers. Based on these marker ions the regio-distribution of the fatty acids esterified to the MAG was determined in a complex lipid sample. The results suggest that EI fragmentation pattern will enable the quality control and monitoring of isomerization processes in unsaturated MAG samples even without commercially available standards.

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