Identification of Diacylglycerols and Triacylglycerols in a Structured Lipid Sample by Atmospheric Pressure Chemical Ionization Liquid Chromatography/Mass Spectrometry

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ABSTRACT: Atmospheric pressure chemical ionization liquid chromatography/mass spectrometry was used in the identification of diacylglycerol and triacylglycerol (TAG) molecular species in a sample of a structured lipid. In the study of acylglycerol standards, the most distinctive differences between the diacylglycerol and TAG molecules were found to be the molecular ion and the relative intensity of monoacylglycerol fragment ions. All saturated TAG ranging from tricaproin to tristearin, and unsaturated TAG including triolein, trilinolein, and trilinolenin, had ammonium adduct molecular ions $[M + NH_4]^+$. Protonated molecular ions were also produced for TAG containing unsaturated fatty acids and the intensity increased with increasing unsaturation. Diacylglycerol fragment ions were also formed for TAG. The ammonium adduct molecular ion was the base peak for TAG containing polyunsaturated fatty acids, whereas the diacylglycerol fragment ion was the base peak for TAG containing saturated and monounsaturated medium- and long-chain fatty acids; the relative intensities of the ammonium adduct molecular ions were between 14 and 58%. The most abundant ion for diacylglycerols, however, was the molecular ion $[M - 17]^+$, and the relative intensity of the monoacylglycerol fragment ion was also higher than that for TAG. These distinctive differences between the diacylglycerol and TAG spectra were utilized for rapid identification of the acylglycerols in the sample of a structured lipid.

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KEY WORDS: APCI LC/MS, diacylglycerol, fragmentation, identification, interesterification, molecular ion, structured lipids, triacylglycerol.

Lipase-catalyzed interesterification has been used in lipid modification for the production of different kinds of lipids according to specific requirements, for instance the enrichment of triacylglycerols (TAG) with polyunsaturated fatty acids, especially essential fatty acids (1,2), and the incorporation of medium- or short-chain fatty acids into TAG (3–5). Since the interesterification alters the fatty acid composition and distribution in the acylglycerols, a group of new TAG is produced in the interesterification. Diacylglycerols are formed as the intermediates and are also found in the interesterified products; the level of diacylglycerols varied in different interesterification processes or under different reaction parameters (6,7).

Reversed-phase–high-performance liquid chromatography (RP–HPLC) is a practical method for the separation of TAG molecular species according to the differences in carbon numbers and unsaturation levels of fatty acids in TAG (8–12). Equivalent carbon number (ECN) or partition number, a summary of both the carbon number and the degree of unsaturation of fatty acids, is commonly used for the tentative identification of TAG (8–10,13,14). However, it was difficult to distinguish diacylglycerols from TAG on the RP–HPLC using the ECN. Even though it is possible to separate and identify the diacylglycerol and TAG molecular species by thin-layer chromatography followed by HPLC fractionation and analysis using gas–liquid chromatography (9), the procedure is tedious and seldom used.

Mass spectrometry (MS) is a sensitive method for molecular analysis, which can provide information on the molecular mass and structure of the analyte. It has been used for the identification of TAG. For instance, a quadrupole mass spectrometer coupled to RP–HPLC *via* a direct liquid inlet interface has been used for the identification of TAG in randomized butteroil (15); and HPLC-desorption chemical ionization tandem MS has been used for the identification of milk fat TAG (16). Recently, the atmospheric pressure chemical ionization (APCI) MS has also been used in the analysis of TAG (17,18). Both protonated molecular ions $[M + H]^+$ and diacylglycerol fragment ions $[MH - RCOOH]^+$ were found in most of the TAG, whereas only diacylglycerol fragment ions were observed in the spectra of TAG only containing saturated fatty acids (17,18).

To our knowledge, no study on the identification of diacylglycerol molecular species with APCI liquid chromatography (LC)/MS has been reported, nor has any study been presented on the identification and classification of diacylglycerol and TAG molecular species in structured lipids by LC/MS. In the present study, we investigated the mass spectra of TAG and

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diacylglycerol standards on APCI LC/MS and optimized the MS system so we could observe both molecular ions and fragment ions for both TAG and diacylglycerols. We found distinctive differences between the TAG and diacylglycerol spectra, which made it possible for fast identification and classification of the diacylglycerols and TAG in the interesterified product.

EXPERIMENTAL PROCEDURES

Reagents and solvents. All reagents and solvents were of analytical or chromatographic grade. Acetonitrile, hexane, and isopropanol were from BDH Laboratory Supplies (Poole, England); ammonium acetate was from Merck (p.a. Darmstadt, Germany).

Interesterified products. The structured lipid sample was produced by the interesterification between rapeseed oil (Århus Olie A/S, Århus, Denmark) and capric acid (99.6%; Henkel Kimianika Sdn. Bhd., Selangor, Malaysia) in a stirred-tank reactor. The interesterification procedure and parameters have been described previously (19). The interesterified product is a mixture of TAG, diacylglycerols, and free fatty acids. The fatty acid profile of acylglycerols is listed in Table 1. The structured lipid sample (interesterified product) was dissolved in chloroform (50 mg/mL), and 5-μL aliquots were injected for HPLC and LC/MS analysis.

HPLC. The diacylglycerol and TAG molecular species were separated on a Supelcosil LC-C18 column (l = 25 cm, i.d. = 4.6 mm, particle size = 5 μm; Supelco, Inc., Bellefonte, PA) with a binary solvent system of acetonitrile (solvent A) and isopropanol/hexane (solvent B, 2:1, vol/vol) (9). The column was fitted into a JASCO HPLC system, consisting of an AS-950 autosampler, two PU-980 pumps, and an HG-980-30 solvent-mixing module (Tokyo, Japan). A SEDEX 55 evaporative light-scattering detector (ELSD) (SEDERE, Alfortville, France) was used and the operating temperature and pressure were 40°C and 2.2 bar, respectively.

APCI LC/MS. The diacylglycerols and TAG were separated as described above. The column was fitted into an HP 1100 Series LC/MSD system, consisting of a quaternary pump, a vacuum degasser, an autosampler, a diode array detector, and an MS detector (Hewlett-Packard, Waldbronn, Germany). All of the column effluent was admitted to the mass spectrometer. The APCI was used in the positive mode, and the solvent vapor acted as the reagent gas. Ammonia acetate (50 mM in isopropanol/water = 1:1) was supplied after the separation column at a flow rate of 50 μL/min. The capillary voltage was 3000 V, the vaporizer temperature was 325°C, and nebulizer gas (nitrogen) pressure was 60 psi. The heated nitrogen drying gas temperature and flow rate were 350°C and 4.0 L/min, respectively. Full mass spectra were taken in the mass range of 65–950, and the step size was 0.1 *m/z*. System control and data evaluation were conducted using HP ChemStation.

Standard solutions. Monoacid TAG standard triacetin, tributyrin, tricaproin, tricaprylin, tricaprin, trilaurin, trimyristin, tripalmitin, tristearin, triolein, trilinolein, and trilinolenin were used for preparing the TAG standard mixture. The mixture was prepared by dissolving the TAG in chloroform, and the concentration was about 2.5 mg/mL for each TAG. The purity of all the TAG standards was 99% except that trilinolenin was 98% (Sigma Inc., St. Louis, MO).

Another standard mixture was prepared by dissolving palmitic acid, 2-monopalmitin, 1,3-dipalmitin, and tripalmitin in chloroform; concentrations were about 2.5 mg/mL for each component. All standards were from Sigma, and their purity was 99%.

TABLE 1
Major Fatty Acids (mol%) in TAG of Structured Lipids[a]

Fatty acid	TAG	*sn*-2
C10:0	58.8	7.8
C16:0	1.3	
C18:0	0.4	
C18:1n-9	20.9	43.6
C18:2n-6	12.4	32.8
C18:3n-3	6.2	15.7

[a]Diacylglycerol content of the structured lipid sample was 10.7 wt%. TAG, triacylglycerols.

RESULTS AND DISCUSSION

Optimization of the LC/MS system. The APCI LC/MS system can tolerate a wide range of liquid flow without loss of MS performance, and a splitter is not necessary when a normal flow rate of the mobile phase is used. Therefore, we directly adopted the RP–HPLC method (9) to the APCI LC/MS. Since we used a nonaqueous system, the intensity of the total ion current was relatively low. To increase the ion current without any change in the separation, we used ammonium acetate as the postcolumn additive. APCI is considered a solvent-mediated chemical ionization (CI); therefore, the most abundant ion in the reagent gas is formed from the protonated mobile-phase constituent with the highest proton affinity (20). The proton affinity of the mobile phase we used was lower than that of ammonium acetate; therefore, the observed ions are primarily ammonium-related.

Important experimental parameters of solvent-mediated CI are the ion-source pressure and temperature, and the solvent composition (20). APCI uses a corona needle discharge to impart a charge onto vaporized molecules that are sprayed from a capillary inlet. These ions are guided into the vacuum region of the mass spectrometer through a dielectric capillary. We optimized the corona discharge current and the capillary voltage (Vcap) since they affect the ionization stability and sensitivity. The Vcap and corona discharge current were varied between 2,000 and 5,000 V and 2 and 8 μA, respectively. Stable results were obtained when the Vcap and corona discharge current were 3,000 V and 5 μA, respectively, and they were applied in the following studies.

In order to elucidate TAG structures, both molecular ions and fragment ions are required; the former provides the information on the molecular mass and the latter provides the in-

formation about the acyl groups in the TAG. Since APCI is a soft ionization technique, it normally produces a large number of molecular ions in the positive mode such as protonated molecular ions and ammonium adduct molecular ions. Fragment ions, however, can be produced deliberately by collision-induced dissociation (CID), and the efficiency of the ion-molecule collisions can be enhanced by applying a small potential difference between the two ion sampling plates (the nozzle and the skimmer) (20). Using high potentials, CID can provide a fingerprint spectrum that is characteristic of the molecular structure just as an electron impact spectrum is; however, it will also result in the loss of the molecular ions. It has been reported previously that no molecular ions for TAG that only contained saturated fatty acids were observed in the analysis of TAG with APCI LC/MS (17,18), which could be due to a relatively high potential applied for CID.

In this study, we aimed to obtain both molecular ions and fragment ions for acylglycerols, so the potential for the CID (fragmentation potential) was selected. A mixture of TAG standards was injected *via* flow injection analysis (FIA) and the fragmentation potential was varied between 60 and 180 V. The optimal potential for ammonium adduct molecular ion of triolein was between 120 and 150 V, whereas the optimal potential for forming the ammonium adduct molecular ions for tricaproin was much lower (60 V) (Fig. 1). This result indicates that the fragmentation potential should be reduced with the decrease in chain length of acyl groups in the TAG to get the optimal transmission of molecular ions. Low fragmentation potential, however, will result in low-level fragmentation, which consequently results in difficulties in structure elucidation. Since the structured lipid sample contained only long- and medium-chain fatty acids, we selected a fragmentation potential of 120 V in the study of TAG standards and the sample of structured lipid.

Analysis of TAG standards with APCI LC/MS. Based on our experience with RP–HPLC in the analysis of TAG, we wanted to compare the HPLC chromatograms from HPLC with ELSD and LC/MS. A homolog series of monoacid TAG was studied by both RP–HPLC with ELSD and APCI LC/MS. Triacetin could not be detected by the ELSD (Fig.

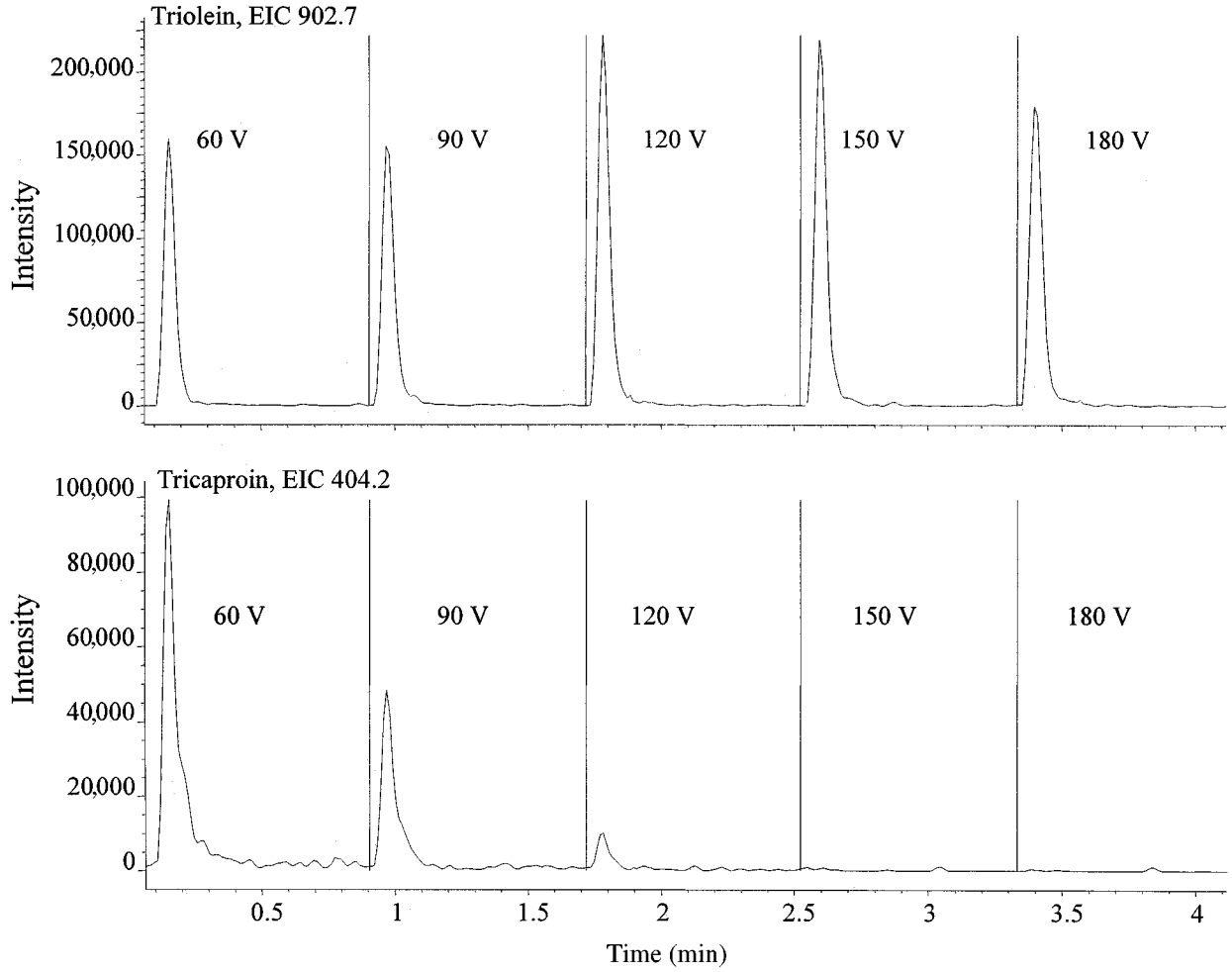

FIG. 1. Selection of the fragmentation voltage in the study of triacylglycerol (TAG) standards with positive atmospheric pressure chemical ionization liquid chromatography–mass spectrometry (APCI LC/MS). Extracted ion chromatogram for ammonium adduct molecular ion of triolein (*m/z* 902.7) and tricaproin (*m/z* 404.2).

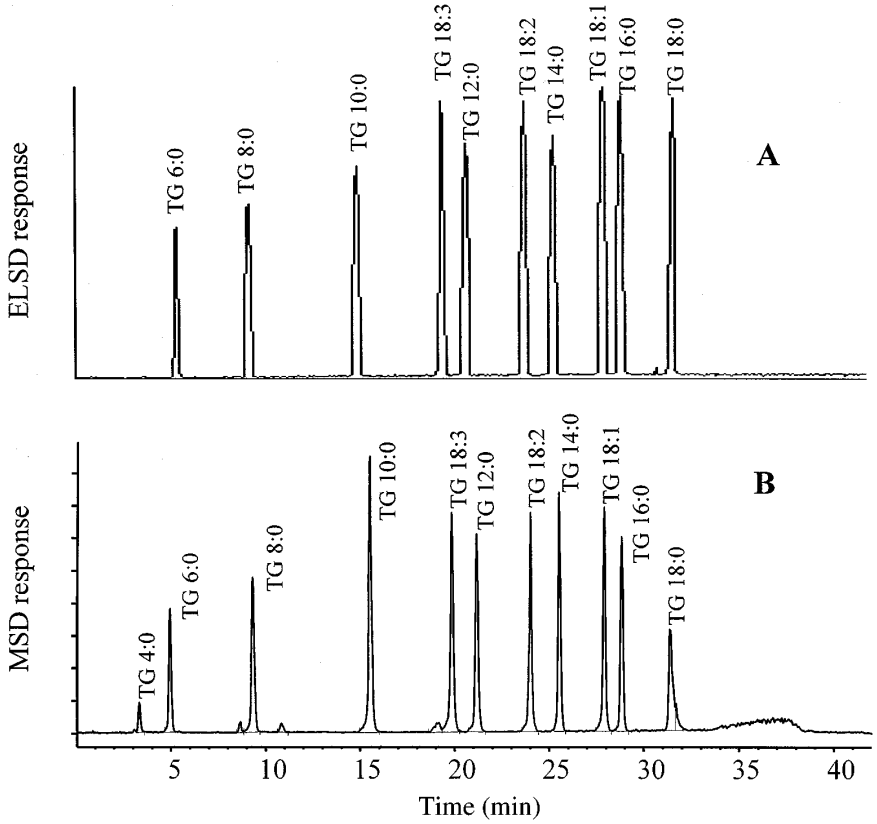

FIG. 2. The high-performance liquid chromatography (HPLC) chromatogram (A) and total ion current (TIC) chromatogram (B) of the TAG standards. The separation was performed on an LC-C18 (Supelco, Bellefonte, PA) column. Evaporative light-scattering detection was used in reversed-phase (RP)–HPLC, temperature 40°C, air pressure 2.2 bar. APCI LC/MS was operated in the positive mode. See Figure 1 for other abbreviations.

2A), and the response of tributyrin was very low even though more than 10 μg was injected. This result might indicate that the size of a molecule affects the size of the mist, and thus affects the scattered light intensity. Accordingly, small molecules could not produce any ELSD response; for instance, the solvent peak in the ELSD is not observed because of the small molecular size of the solvents.

The total ion current chromatogram of APCI LC/MS (Fig. 2B) was similar to the chromatogram obtained on RP–HPLC with ELSD. No triacetin was detected by MS, and only a small amount of tributyrin was detected. It has been reported that the properties of the analyte affect the gas-phase ion production in a number of ways, e.g., in its solvophobicity or the tendency to reside at the droplet surface. For instance, in the analysis of quaternary ammonium compounds, the selected ion current increases with increasing chain length of the alkyl groups of the quaternary ammonium salt since an increase in chain length is accompanied by an increase in solvophobicity (20). Therefore, the smaller the molecule is, the higher the solvophilicity will be, and the harder it will be to remove the analyte from the droplet. This may explain why we could not observe the small molecules such as triacetin and the low MS response for tributyrin.

The relative response factors of ELSD and MS for different TAG were calculated based on the response of trilaurin (Table 2). Since ELSD and MS are based on different detection principles, the response factors are also different. The relative response factors of ELSD for different TAG increased

TABLE 2
Relative Response Factors of ELSD and MS for TAG Standards[a]

TAG	ECN	Retention time (min)	Relative RF	
			APCI LC/MS	HPLC/ELSD
Tributyrin	12	3.35	0.11	0.06
Tricaproin	18	4.96	0.50	0.62
Tricaprylin	24	9.33	0.81	0.66
Tricaprin	30	15.53	1.34	0.79
Trilinolenin	36	19.86	1.07	0.91
Trilaurin	36	21.17	1.00	1.00
Trilinolin	42	24.02	0.89	1.06
Trimyristin	42	25.54	1.00	1.10
Triolein	48	27.92	0.76	1.03
Tripalmitin	48	28.84	0.90	1.17
Tristearin	54	31.39	0.76	1.14

[a]ELSD, evaporative light-scattering detector; MS, mass spectrometer; ECN, equivalent carbon number; RF, response factor; APCI LC/MS, atmospheric pressure chemical ionization liquid chromatography–mass spectrometry; HPLC/ELSD, high-performance liquid chromatography ELSD. See Table 1 for other abbreviation.

with the increase of ECN values, and the variation was most significant for TAG containing short- and medium-chain fatty acids (Table 2). A large variation between the response factors of MS for different TAG was observed (Table 2). With the increase in chain length of acyl groups, the intensity of the total ion current of TAG increased significantly from tributyrin to tricaprin, whereas it decreased from trimyristin to tristearin. The unsaturation level of the acyl groups also affected the response factors of MS; for instance, the value of relative response factor of MS increased in the order of triolein, trilinolein, and trilinolenin. The variation in relative response factors among different TAG implies that the application of individual response factors is necessary for accurate quantification of TAG.

For all TAG standards, we could observe the ammonium adduct molecular ions $[M + NH_4]^+$, diacylglycerol fragment ions $[MH - RCOOH]^+$, and monoacylglycerol fragment ions $[MH - 2RCOOH + 18]^+$, but the intensities of monoacylglycerol ions were very low (Table 3). The diacylglycerol fragment ion was the base peak for TAG containing saturated and monounsaturated fatty acids, whereas the ammonium adduct molecular ion was the base peak for TAG containing polyunsaturated fatty acids. The relative intensity of the ammonium adduct molecular ions was in the range of 14–60% for TAG containing medium- and long-chain fatty acids. Lower intensity of ammonium adduct molecular ions was observed for tributyrin and tricaproin (Table 3) due to the fragmentation potential being too high for those small molecules. Fatty acid fragment ions $[RCO]^+$ were formed from the TAG containing short- and medium-chain fatty acids, and their intensities decreased significantly with the increase of chain length of fatty acids (Table 3). The protonated molecular ion $[M + H]^+$ was also formed for TAG containing unsaturated fatty acids, and the intensity increased with increasing unsaturation level, while the intensity of the fragment ions decreased (Fig. 3). The ratios between the intensities of protonated molecular ion and ammonium adduct molecular ion may be used to predict the unsaturation level of TAG, but further study is necessary in order to utilize this possible function.

In comparison with the results reported previously (17,18), that only diacylglycerol fragment ions were found in the spectra of TAG containing saturated fatty acids, our present study demonstrated the possibilities to obtain both ammonium adduct molecular ions and diacylglycerol fragment ions for TAG containing only saturated fatty acids by APCI LC/MS. By knowing both the molecular ions and fragment ions of unknown TAG, their structures can be easily elucidated.

Analysis of partial acylglycerol standards with APCI LC/MS. In the study of the mass spectra of partial acylglycerols, a mixture of palmitic acid, 2-monopalmitin, 1,3-dipalmitin, and tripalmitin was analyzed under the same condition for the analysis of standard TAG. Only dipalmitin and tripalmitin were detected by MS, indicating that the APCI LC/MS is not sensitive enough for the analysis of monoacylglycerols and free fatty acids; higher sample loading or another ionization source is necessary.

The fragmentograms of dipalmitin and tripalmitin are shown in Figure 4. The most abundant ion for dipalmitin was *m/z* 551 representing the ion $[M - 17]^+$ (Fig. 5), which might be the degradation product of the ammonium adduct molecular ion formed by losing a NH_4OH molecule (Fig. 6). An ammonium adduct molecular ion for dipalmitin (*m/z* = 586) was also observed by ion extraction, but the intensity was very low (about 0.2% of that of base peak). The monoacylglycerol fragment ion *m/z* 313 was also detected, and its intensity was about 56.3% of that of the base peak.

The diacylglycerol fragment ion $[MH - C_{16:0}]^+$ is the base peak for tripalmitin. The relative intensity of the ammonium adduct molecular ion $[M + NH_4]^+$ and the monoacylglycerol fragment ions $[MH - 2C16:0 + 18]^+$ were 42.3 and 4.5%, respectively. There were significant differences between the molecular ions and the monoacylglycerol fragment ions for diacylglycerol and TAG. The former had the special molecular ion $[M - 17]^+$ and the relative intensities of the monoacylglycerol fragment ions were high, whereas the latter had the ammonium adduct molecular ions $[M + NH_4]^+$ and the relative intensities of the monoacylglycerol fragment ions were low. Those distinct differences can be used as an important

TABLE 3
The Abundance of Ions Produced from Different Monoacid TAG in APCI LC/MS[a]

		t_R	$[M + NH_4]^+$		$[M + H]^+$		$[MH - RCOOH]^+$		$[MH - 2RCOOH + 18]^+$		$[RCO]^+$	
TAG	ECN	(min)	*m/z*	Abun.	*m/z*	Abun.	*m/z*	Abun.	*m/z*	Abun.	*m/z*	Abun.
Tributyrin	12	3.35	320	0.6			215	100	145	0.6	71	84
Tricaproin	18	4.96	404	2.9			271	100	173	0.5	99	30
Tricaprylin	24	9.33	488	14			327	100	201	0.7	127	11
Tricaprin	30	15.53	572	14			383	100	229	0.9	155	4.3
Trilaurin	36	21.17	656	49			439	100	257	1.8	183	0.6
Trimyristin	42	25.54	740	52			495	100	285	2.4	211	0.6
Tripalmitin	48	28.84	824	35			551	100	313	6.1	239	0.2
Tristearin	54	31.39	908	50			607	100	341	4.3		
Triolein	48	27.92	902	58	885	4.4	603	100	339	5.5		
Trilinolein	42	24.02	896	100	879	44	599	41	337	3.8		
Trilinolenin	36	19.86	890	100	873	69	595	27	335	3.6		

[a]Abun., abundance; see Tables 1 and 2 for other abbreviations.

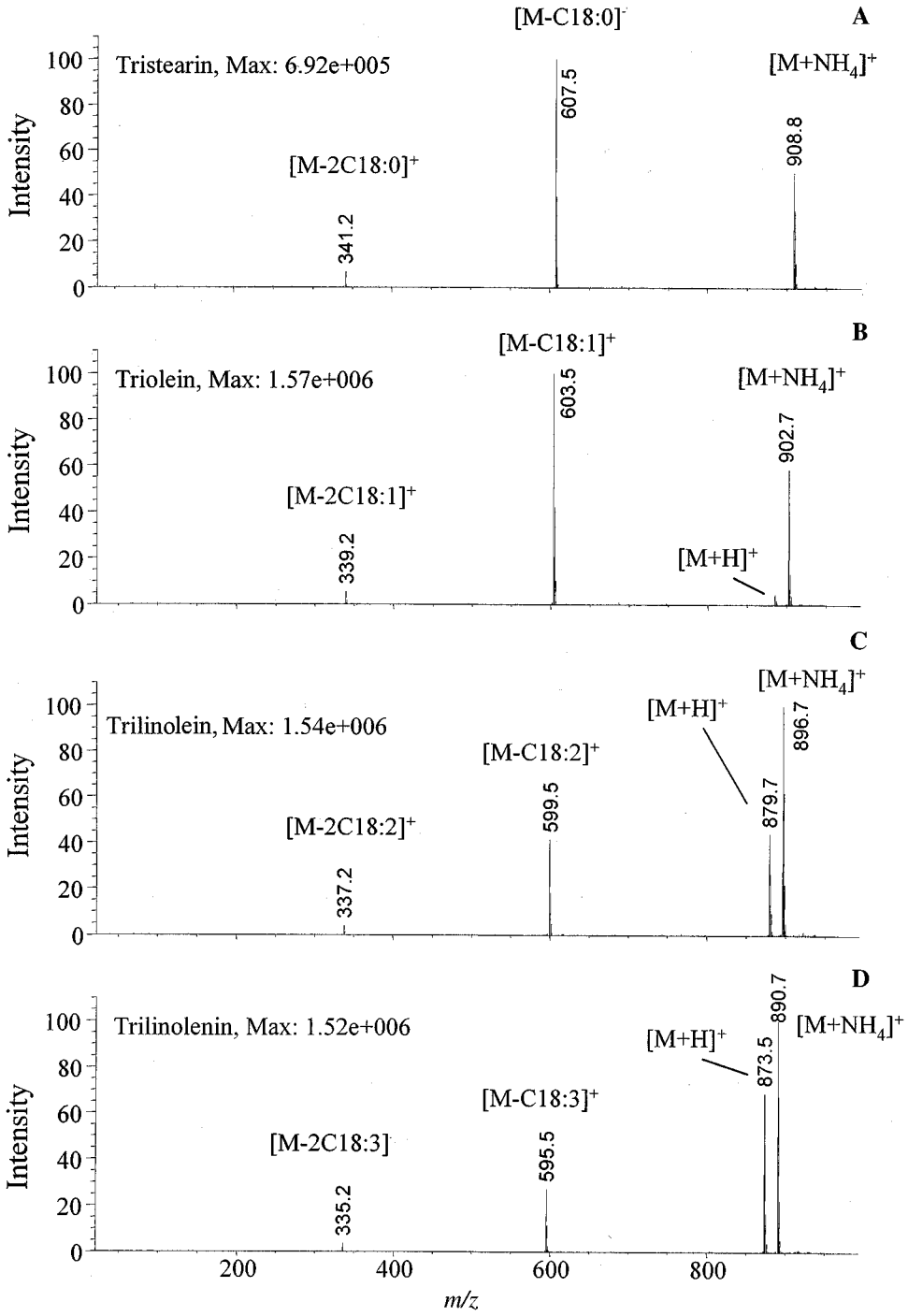

FIG. 3. APCI mass spectra of monoacid TAG containing fatty acids representing different levels of unsaturation. (A) Tristearin (TG18:0), (B) triolein (TG18:1), (C) trilinolein (TG18:2), and (D) trilinolenin (TG18:3). See Figure 1 for abbreviations.

tool for identifying diacylglycerol and TAG molecular species in structured lipid samples.

Identification of molecular species in structured lipid sample. Diacylglycerols are produced as the intermediate during the lipase-catalyzed interesterification; since hydrolysis is the primary step for the interesterification, the level of diacylglycerols was much lower than the level of TAG. It was difficult to identify if the compounds eluted from the RP–HPLC column were TAG or diacylglycerols for the structured lipid sample, since the separation was based only on the ECN. To assist in the identification, the structured lipid sample was analyzed with APCI LC/MS under selected conditions. Figure 7 shows the total ion current chromatogram of the structured lipid sample.

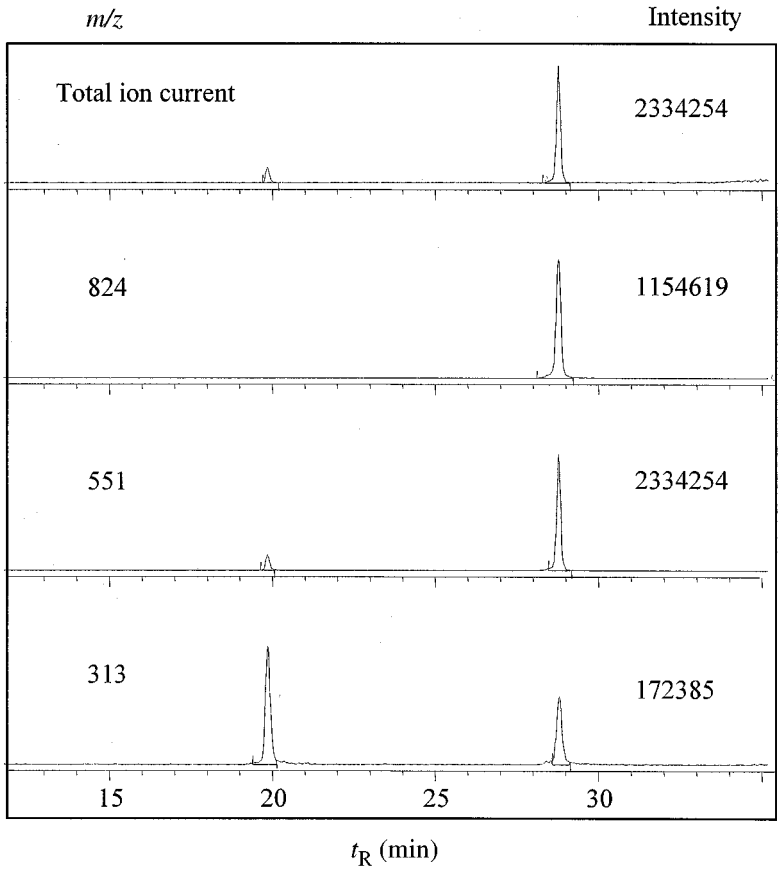

FIG. 4. APCI LC/MS fragmentograms of tripalmitin and dipalmitin, showing traces of the total ion current; *m/z* 824 characteristic of tripalmitin ($[M + HN_4]^+$); *m/z* 551 characteristic of both tripalmitin ($[MH - C16:0]^+$) and dipalmitin ($[M - 17]^+$); and *m/z* 313 characteristic of monoacylglycerol fragment ions for both di- and tripalmitin. See Figure 1 for abbreviation.

The relatively high intensity of the monoacylglycerol fragment ions and the special molecular ion $[M - 17]^+$ were used to identify the diacylglycerols in the structured lipid sample. There were no ammonium adduct molecular ions observed for the acylglycerols labeled as peaks 1 to 5 in the total ion chromatogram shown in Figure 7, and the relative intensity of monoacylglycerol fragment ions was high in comparison with TAG. The most abundant ion was the special molecular ion $[M - 17]^+$ for those peaks (Fig. 8); therefore, they were identified as diacylglycerols. The molecular masses of the diacylglycerols were calculated as 400, 400, 506, 508, and 510; and they represent the diacylglycerols 10:0/10:0, 10:0/10:0, 10:0/18:3, 10:0/18:2, and 10:0/18:1, respectively. In our previous study, we could separate 1,2- and 1,3-diacylglycerols on RP–HPLC (9); therefore, the two dicaprins identified in the structured lipid sample may represent the positional isomers 1,3-dicaprin and 1,2-dicaprin.

We observed ammonium adduct molecular ions for the other acylglycerols in the structured lipid sample, and we also observed protonated molecular ions. Based on the knowledge we obtained previously from the standards that APCI LC/MS did not produce or produced only very small amounts of ammonium adduct ions for diacylglycerols, those compounds were therefore suggested to be TAG. They were identified from their ammonium adduct molecular ions and their diacylglycerol fragment ions, and the results are listed in Table 4.

According to the spectra of the TAG in the structured lipid sample, the base peak for different kinds of TAG can be summarized as the following: monoacid TAG had a diacylglycerol fragment ion as the base peak; diacid TAG containing long-chain fatty acids or one medium-chain and two long-chain fatty acids had an ammonium adduct molecular ion as the base peak, but the TAG containing two medium-chain fatty acids had a diacylglycerol fragment ion as the base peak, and triacid TAG had an ammonium adduct molecular ion or protonated molecular ion as the base peak. Figure 9 shows the mass spectra of diacid and triacid TAG identified in the structured lipid sample. Monoacylglycerol fragment ions were also observed for monoacid TAG, but the relative intensity was very low.

In the mass spectrum of TAG 10:0/18:3/10:0 (peak 7), we also found the *m/z* 601 ion, which was the special molecular ion $[M - 17]^+$ of the diacylglycerol 18:1/18:2, indicating co-elution of the diacylglycerol and the TAG. Similarly, we also found co-elution of the TAG 10:0/18:2/10:0 with the diacyl-

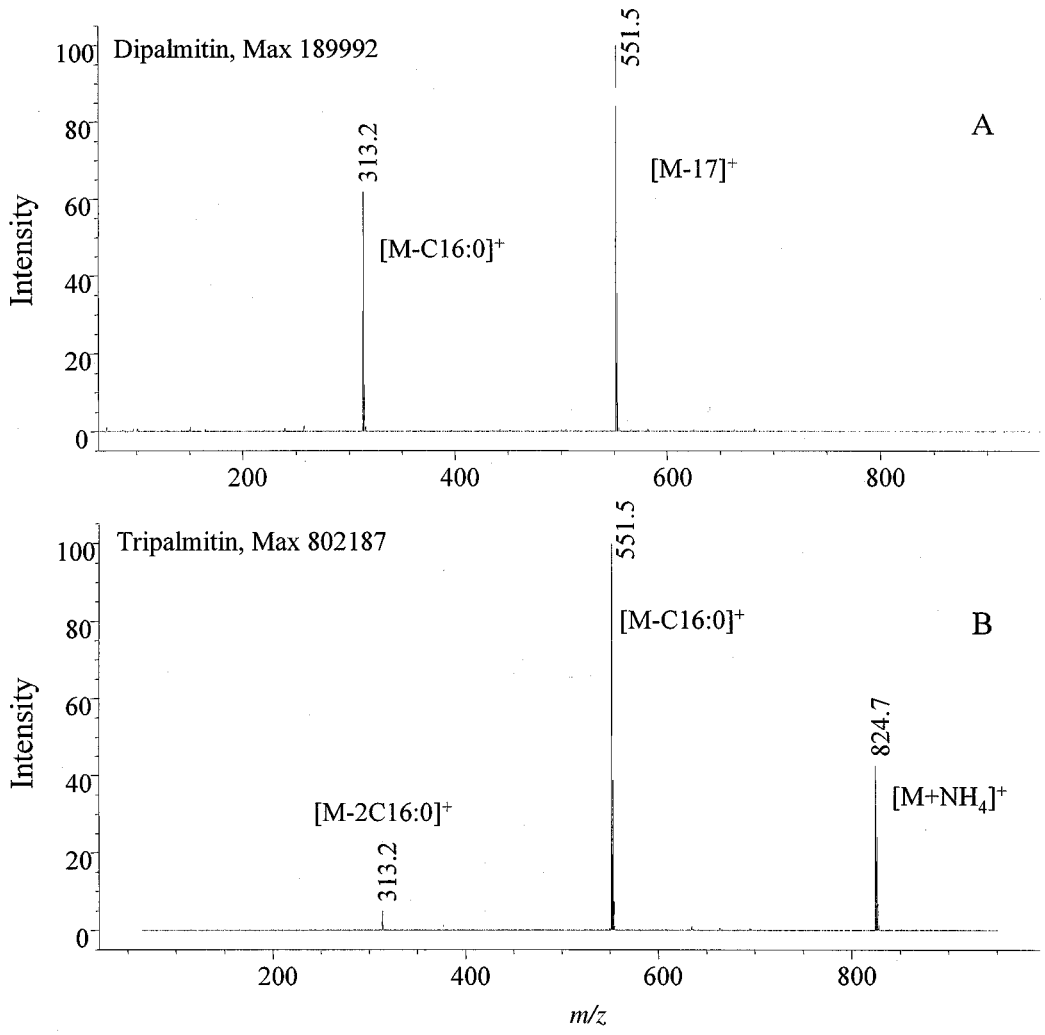

FIG. 5. Positive APCI mass spectra of (A) dipalmitin and (B) tripalmitin. See Figure 1 for abbreviation.

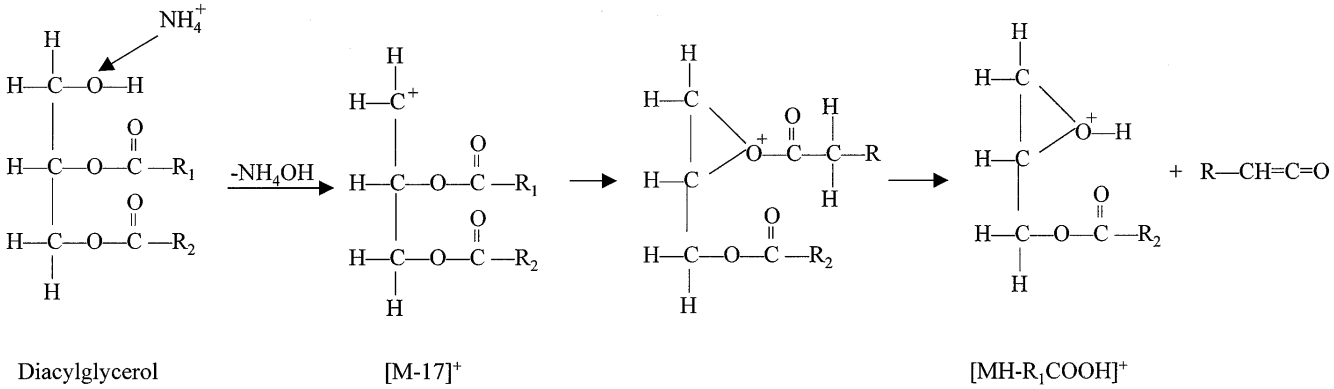

FIG. 6. Suggested ion formation and fragmentation pathway for diacylglycerols. See Figure 1 for abbreviation.

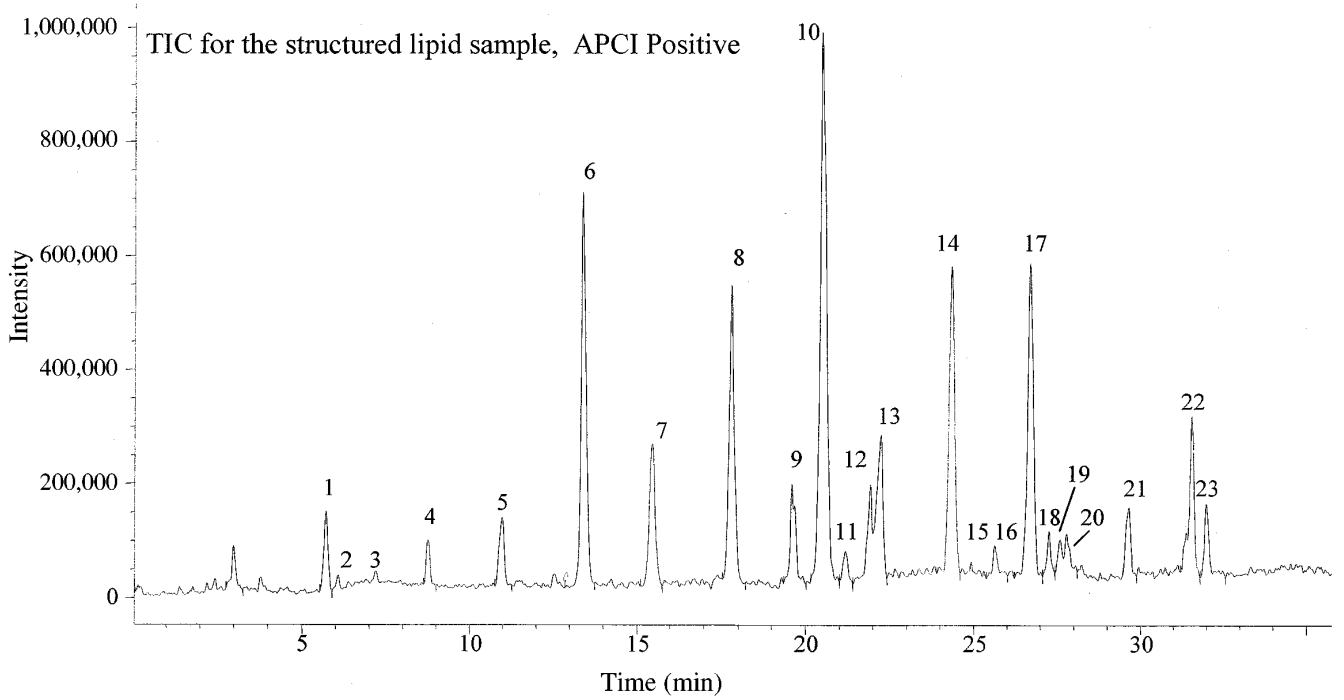

FIG. 7. The TIC chromatogram of the structured lipid sample, APCI MS in positive mode. Peaks 1 to 5 represent diacylglycerols 10:0/10:0, 10:0/10:0, 10:0/18:3, 10:0/18:2 and 10:0/18:1, respectively. Peaks 6 to 23 represent TAG 10:0/10:0/10:0, 10:0/18:3/10:0, 10:0/18:2/10:0, 10:0/18:2/18:3, 10:0/18:1/10:0, 10:0/16:0/10:0, 10:0/18:2/18:2, 10:0/18:3/18:1, 10:0/18:2/18:1, 10:0/18:2/16:0, 18:3/18:2/18:1, 10:0/18:1/18:1, 16:0/18:1/10:0, 18:2/18:2/18:1, 18:1/18:1/18:3, 18:2/18:1/18:1, 18:1/18:1/18:1, and 18:1/18:1/16:0, respectively. See Figures 1 and 2 for other abbreviations.

TABLE 4
Major Ions of TAG in the Structured Lipid Sample and Their Abundances

t_R			$[M + H]^+$		$[M + NH_4]^+$		$[MH - RCOOH]^+$					
(min)	TAG	MW	*m/z*	Inten.	*m/z*	Inten.	*m/z*	Inten.	*m/z*	Inten.	*m/z*	Inten.
13.37	10:0/10:0/10:0	554			572	286166	383	812519				
15.47	10:0/18:3/10:0	660	661	38816	678	265274	489	271742	383	101719	601.5[a]	11058
17.80	10:0/18:2/10:0	662	663	4346	680	432311	491	622488	383	131239		
19.59	10:0/18:2/18:3	768	769	2E+05	786	142848	597	47976	489	66974	491	56784
20.49	10:0/18:1/10:0	664			682	610051	493	1E+06	383	388214		
21.18	10:0/16:0/10:0	638			656	60212	467	85392	383	45980		
21.91	10:0/18:2/18:2	770	771	47783	788	88740			491	88740		
22.23	10:0/18:3/18:1	770	771	31954	788	177520	599	69233	493	102962	489	71008
24.33	10:0/18:2/18:1	772	773	22051	790	624256	601	349170	493	344067	491	3E+05
24.90	10:0/18:2/16:0	746			764	34558	575	7593	491	14877	468	8988
25.62	18:3/18:2/18:1	878	879	39238	896	99920	601	16986	599	19854	597	22051
26.69	10:0/18:1/18:1	774	775	10778	792	623360	603	220864	493	512067		
27.26	16:0/18:1/10:0	748	749	1072	766	134016	577	53165	493	63155	467	10778
27.57	18:2/18:2/18:1	880	881	39760	898	107200	599	27622	601	51514		
27.78	18:1/18:1/18:3	880	881	16220	898	63742	599	20773	603	15366		
29.66	18:2/18:1/18:1	882	883	18721	900	162752	601	168690	603	107040		
31.54	18:1/18:1/18:1	884	885	6614	902	127571	603	353536				
31.97	18:1/18:1/16:0	858	859	1210	876	28672	603	24808	577	56237		

[a]*m/z* 601 is the special molecular ion $[M - 17]^+$ of the diacylglycerol 18:1/18:2, which co-eluted with the triacylglycerol 10:0/18:3/10:0. Inten., intensity; see Table 1 for other abbreviation.

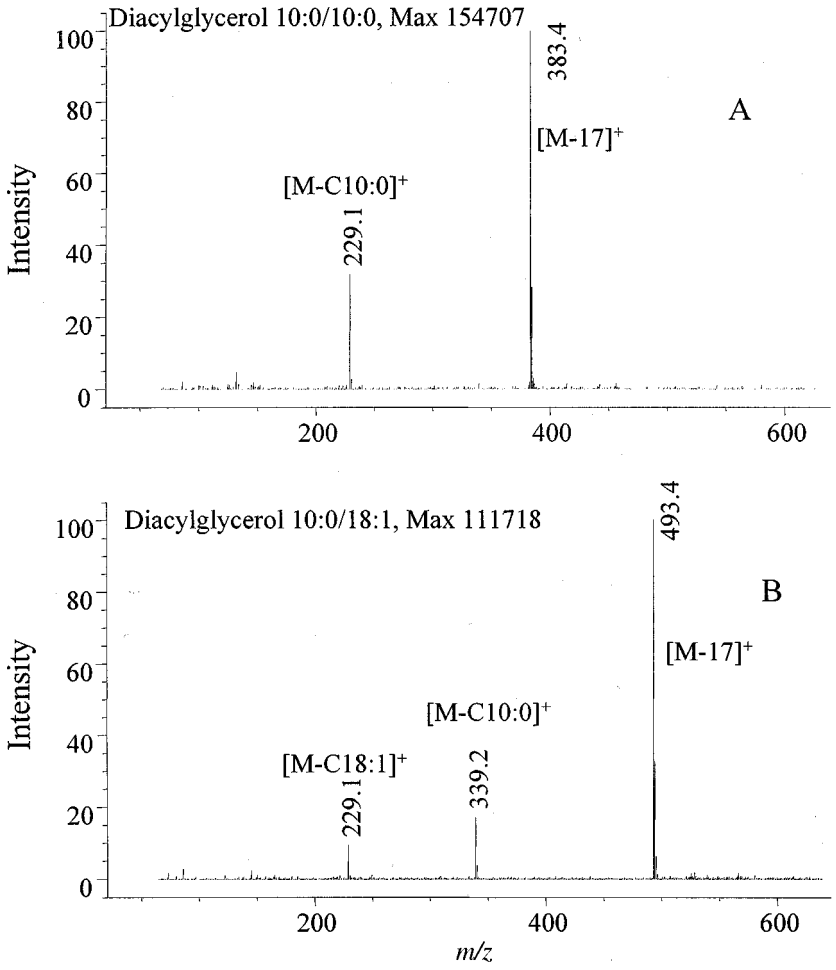

FIG. 8. APCI mass spectra of diacylglycerols (A) 10:0/10:0 and (B) 10:0/18:1 identified in the structured lipid sample (peaks 1 and 5 in Fig. 7). See Figure 1 for abbreviation.

glycerol 18:1/18:1, which also produced the special molecular ion (*m/z* 603).

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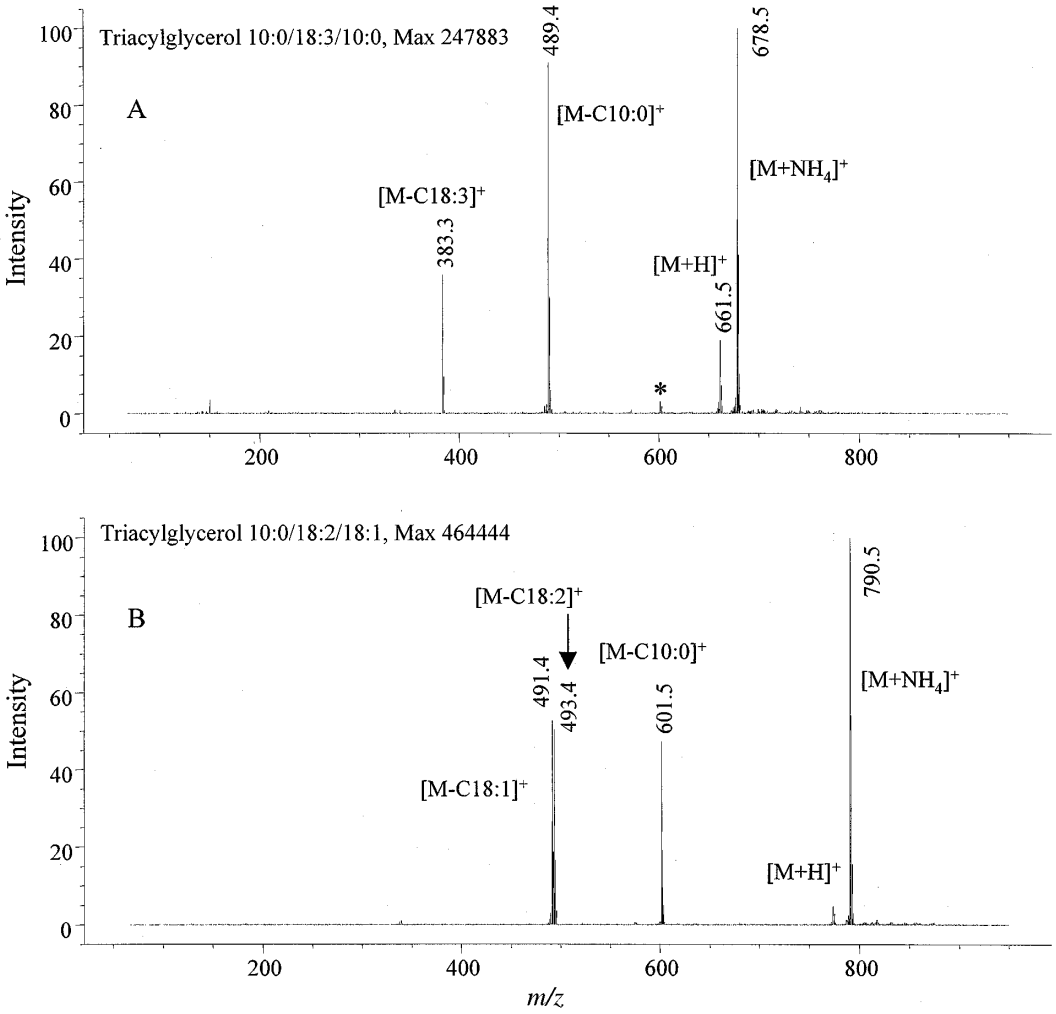

FIG. 9. APCI mass spectra of TAG (A) 10:0/18:3/10:0 and (B) 10:0/18:2/18:1 identified in the structured lipid sample (peaks 7 and 14 in Figure 7). See Figure 1 for abbreviations.

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