Characterization of Peanut Oil Triacylglycerols by HPLC, GLC and EIMS

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Twenty-three peanut oil triacylglycerols have been characterized by liquid chromatography, gas chromatography and electron impact mass spectrometry. High resolution was achieved using two 8-cm × 6.2-mm reverse phase columns in series, and 20 of the triacylglycerols were separated in an analysis time of less than 45 min. Triacylglycerols were identified by analyzing each liquid chromatography fraction for carbon number, fatty acid composition and mass fragmentation pattern. The combined application of these methods permitted the identification of triacylglycerols representing combinations of all of the fatty acids present in peanut oil.

Natural fats and oils are complex mixtures of triacylglycerols, and the number of possible molecular species and isomers is astronomical. A complete determination can be achieved only by several successive procedures that are tedious and time consuming (1). However, this approach is not practical for the oil industry, for quality control programs, and for many research and development programs.

The development of high efficiency, short 3-μ reverse phase columns has created renewed interest in the area of triacylglycerol separation (2). Liquid chromatography on these columns has resulted in improved resolution and shorter analysis time.

Column and mobile phase selectivities have been studied by several workers (3,4). Singleton and Pattee (5) have both optimized parameters for the analysis of peanut triacylglycerols on 10-μ reverse phase columns and studied the effect of micellar mobile phases on the retention behavior of peanut triacylglycerols (6).

Various detectors have been used in the analysis of triacylglycerols separated by high performance liquid chromatography (HPLC). Payne-Wahl et al. (7) used an infrared detector in combination with gradient elution to demonstrate the effectiveness of this detector in HPLC analysis of triacylglycerols. Singleton and Pattee (5,6) have used a UV detector at 210 nm exclusively in the analysis of triacylglycerols. The UV detector at 210 nm takes advantage of the molar absorptivity of the double bond, resulting in enhanced detection of triacylglycerols with unsaturation. Dong and DiCesare (2) analyzed corn oil, palm oil, olive oil and peanut oil by HPLC using short columns and a refractive index detector. Triacylglycerol carbon number assignment for the HPLC-separated peaks from corn oil, olive oil and palm oil was made using 12 standards and elution patterns from previously published literature. However, this could not be done for peanut oil, because all of the triacylglycerols separated from peanut oil on short 3-μ columns had not yet been identified. Merritt et al. (8) separated 12 triacylglycerols by HPLC and identified 16 peanut triacylglycerols by chemical ionization (CI) mass spectrometry using ammonia gas. However, none of the triacylglycerols characterized from peanut oil contained a C-20:1 or C-24:0 fatty acid moiety.

The objectives of this study were (i) to demonstrate the efficiency of 3-μ reverse phase columns in the

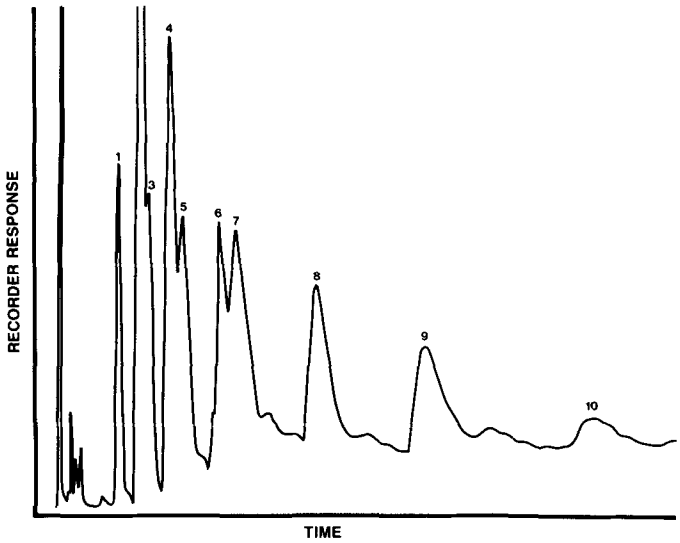

FIG. 1. Preparative chromatogram of peanut oil triacylglycerols on a 10-μ reverse phase column.

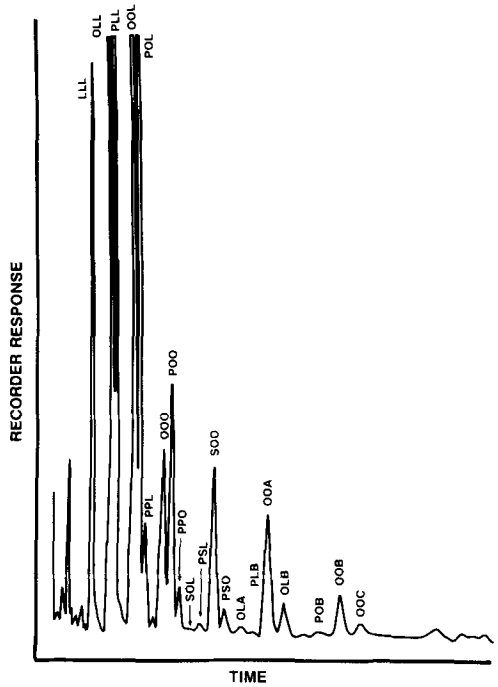

FIG. 2. High resolution chromatogram of peanut oil triacylglycerols on short 3-μ reverse phase columns connected in series. Abbreviations: L, 18:2; O, 18:1; P, 16:0; S, 18:0; A, 20:0; B, 22:0, and C, 24:0.

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separation of peanut triacylglycerols; (ii) to evaluate the effectiveness of the UV detector at 210 nm, and (iii) to evaluate the effectiveness of using gas liquid chromatography (GLC) and electron impact mass spectrometry in the further characterization of the molecular species of triacylglycerols separated by HPLC from peanut oil.

EXPERIMENTAL

Reagents. Triacylglycerol standards were purchased from Supelco Inc. (Bellefonte, Pennsylvania). HPLC grade acetonitrile was purchased from Fisher Scientific (Raleigh, North Carolina). Absolute ethanol was obtained from the Central Stores, North Carolina State University (Raleigh, North Carolina). Peanut oil was obtained by homogenizing peanut seeds three times with chloroform/methanol (2:1, v/v), filtering the homogenate, and removing the solvent by rotary evaporation.

Columns. A MCH-10 (Varian) reverse phase column (C_{18}, 30 cm × 4.0 mm) was used for preparative separation of triacylglycerol fractions. This 10-μ column had an efficiency of 6,076 theoretical plates. High resolution chromatography of peanut triacylglycerols was achieved by connecting two 3-μ reverse phase columns (8 cm × 6.2 mm, Dupont) in series with a combined efficiency of 16,000 theoretical plates.

HPLC of triacylglycerols. Triacylglycerols were separated on reverse phase columns using a Varian 5000 HPLC unit equipped with an automatic injector and 10-μl loop and a UV detector at 210 nm. A 10-μl oil sample (30%) dissolved in chloroform was injected on the column using acetonitrile/absolute ethanol (80:20) as the mobile phase. Each separated peak was collected as it eluted. Repeated injections and collections were necessary to obtain enough material for further analysis. For very high resolution, peanut oil samples were injected onto short 3-μ reverse phase columns in series using acetonitrile/absolute ethanol (30:70). A flow rate of 2 ml/min was used for both preparative and high resolution chromatography. Solvents were removed from the preparative liquid chromatographic fractions by rotary evaporation at 40 C. These fractions were stored at −20 C for further analysis.

GLC of separated HPLC fractions. Each triacylglycerol fraction separated by HPLC was subjected to GLC analysis for determination of both fatty acid composition and carbon number of the triacylglycerols in the fraction. Fatty acid composition of each triacylglycerol fraction separated by HPLC was determined by transesterification (9) and separating the methyl acid esters on an EGSS column (3.05 m × 3.1 mm) (15% on 100/120 mesh Gas-Chrom P) isothermally at 180 C using a Varian 3700 gas chromatograph equipped with dual flame ionization detectors. Carbon numbers of the triacylglycerol fractions were determined by GLC using a Dexsil 300 column (1% on 100/120 Supelcoport) (0.46 m × 3.1 mm) programmed from 240 C to 370 C at 8 C/min or by using the same column isothermally at 290 C. Peaks were tentatively identified by comparison with standard triacylglycerols of known composition.

TABLE 1

Integrated Area for Peanut Oil Triacylglycerols Separated on a 3-μ Reverse Phase Column

Equivalent carbon no.	TG[a]	%	SA[b]
42	LLL	5.82	5.82
44	OLL	26.12	
44	PLL	8.36	34.48
46	OOL	21.53	
46	POL	13.42	
46	PPL	1.86	36.81
48	OOO	4.48	
48	POO	5.62	10.1
50	PPO	0.96	
50	SOO	4.42	
50	PSO	0.60	5.98
52	OOA	4.24	
52	OLB	1.12	5.36
54	OOB	1.42	1.42

[a]L, 18:2; O, 18:1; P, 16:0; S, 18:0; A, 20:0, and B, 22:0.
[b]Summation by equivalent carbon number.

TABLE 2

Fatty Acid Composition and Carbon Number of the Triacylglycerol Fractions of Peanut Oil Separated by HPLC

	HPLC Fractions								
Fatty acid	1	2	3	4	5	6	7	8	9 & 10
16:0	9.45	4.69	13.55	0.92	21.37	48.74	11.00	17.39	15.92
18:0	9.75	0.73	1.19	0.18	2.33	4.02	1.41	5.38	4.14
18:1	0.17	30.88	23.61	60.74	40.58	21.95	68.46	65.11	48.70
18:2	80.64	63.70	61.67	36.55	37.72	25.29	16.01	10.21	12.50
20:0			0.03				0.06	0.98	3.74
20:1				1.62			2.97	1.07	3.70
22:0							0.09		8.49
24:0									2.81
Carbon number	54	54	52	52, 54	52	50,52,54	52,54,56	52,54,56	56,58

Mass spectrometry. Confirmatory identification of the composition of the separated triacylglycerol fractions was made by subjecting a portion of each concentrated fraction to mass spectral analysis using a Hewlett Packard Model 5985B electron impact mass spectrometer. Each fraction was analyzed separately by the insertion probe method (10,11). The probe was programmed ballistically from 25 C to a range of 280 C to 320 C. The output of the mass spectrometer was attached to a computer data collection system. Final triacylglycerol identification for the fractions separated by HPLC was made by using HPLC fractionation data (equivalent carbon number), fatty acid composition and carbon number by GLC, and by molecular ions and fragmentation patterns from mass spectrometry.

RESULTS AND DISCUSSION

HPLC separation of peanut triacylglycerols. The separation of peanut triacylglycerols on a 10-μ C18 reverse phase column (30 cm × 4.0 mm) is shown in Figure 1. Ten distinct fractions were separated using this column. Triacylglycerols are separated on reverse phase columns according to their degree of unsaturation and molecular weight. The degree of unsaturation has the greatest effect on the separation. The presence of double bonds in a triacylglycerol decreases its retention time on reverse phase columns according to the following equation: ECN=CN-2N, where ECN is the equivalent carbon number, CN is the total number of carbons in the triacylglycerol, and N is the number of double bonds in the triacylglycerol (12). Therefore HPLC separation of triacylglycerols is based on the ECN.

HPLC fractions 1, 2, 3, 4 and 5 (Fig. 1) have a high degree of unsaturation and each contains only one triacylglycerol, whereas fractions 6, 7, 8, 9 and 10 contain more than one triacylglycerol and are less unsaturated. The resolution of peanut triacylglycerols on two short 3-μ reverse phase columns connected in series is shown in Figure 2. Twenty of the 23 triacylglycerols identified in this study were resolved by this technique. Integration of the peaks is given in Table 1. Separated peaks with an area below 0.6% were not integrated.

Identification of triacylglycerols. Peak identification in Figures 1 and 2 was based on ECN by HPLC, CN and fatty acid composition, and on mass spectral data. Triacylglycerols analyzed by electron impact mass spectrometry using the probe method exhibit characteristic fragmentation patterns. Ions found in the spectra are the $(RCO)^+$ (the acyl moiety), $(RCO + 74)^1$, $(RCO + 115)$, $(RCO + 128 + 14n)^1$ (the glycerol moiety plus one acyl group), and $(M\text{-}RCO_2)$ (the glycerol moiety plus two acyl groups) (13). In addition, unsaturation results in ions of mass $(RCO\text{-}1)^+$. For example, C-18:2 acyl moiety will form ions at 262 and 263, and a C-18:1 acyl moiety will form ions at 264 and 265.

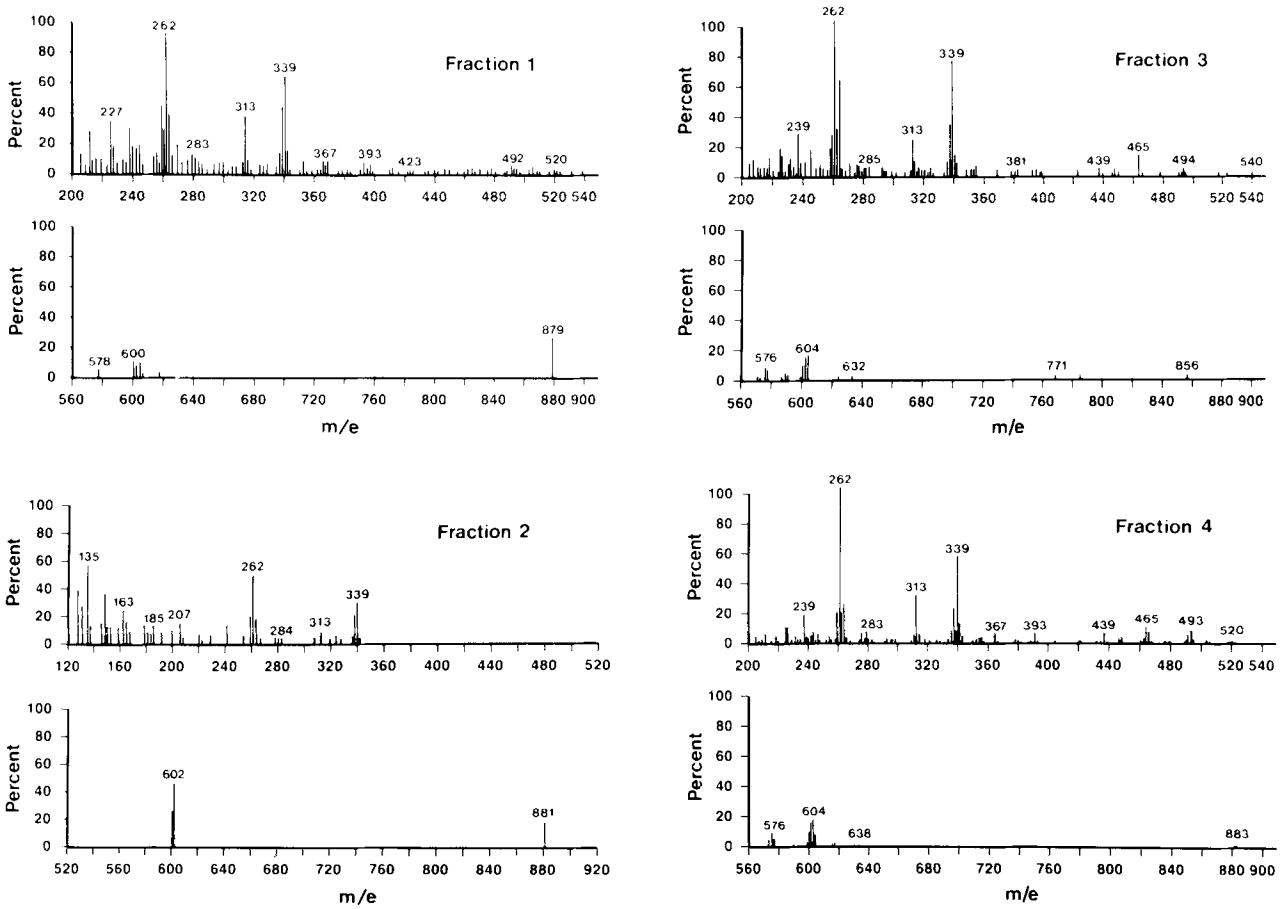

FIG. 3. Mass spectral scans of HPLC fractions 1, 2, 3 and 4.

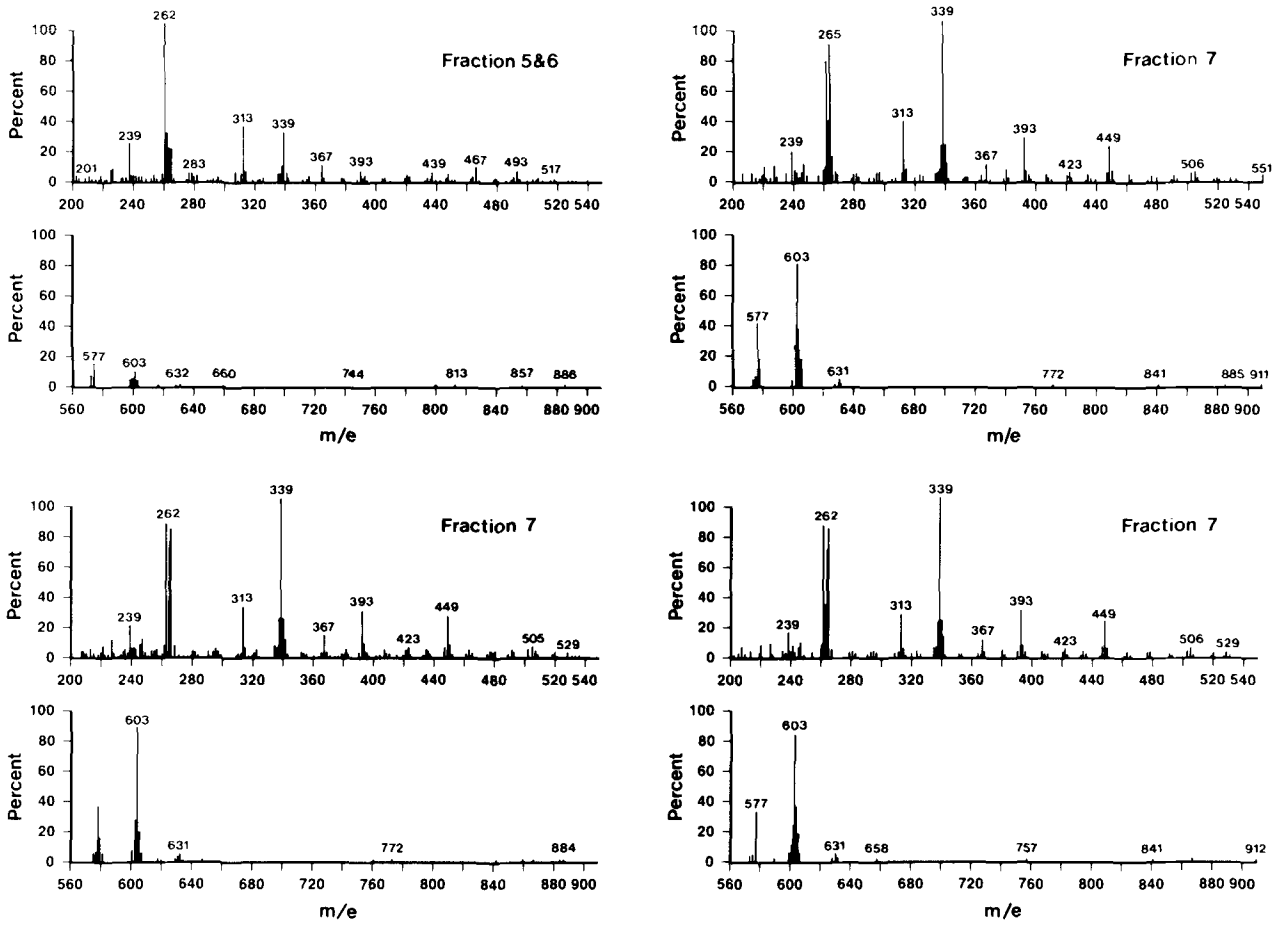

FIG. 4. Mass spectral scans of HPLC fractions 5, 6 and 7.

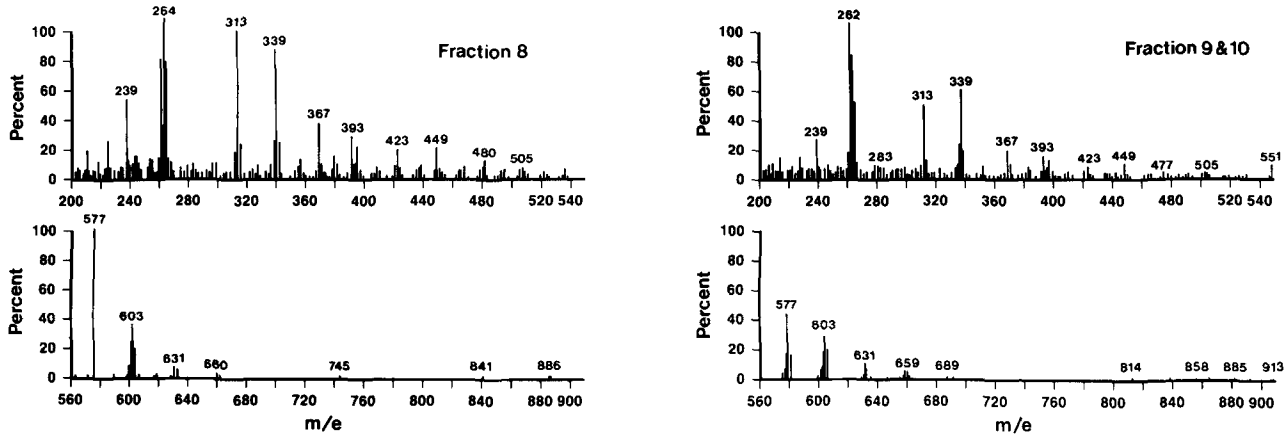

FIG. 5. Mass spectral scans of HPLC fractions 8, 9 and 10.

The mass spectral scan for the first eluting HPLC fraction (Fig. 1) is shown in scan 1 (Fig. 3). The molecular ion at 879 shows the molecular species has the following composition: 18:2 18:2 18:2. Data presented in Table 2 show both the fatty acid composition and a carbon number of 54. This triacylglycerol contains six double bonds and has an ECN of 42. Similar interpretations were made for fractions 2, 3 and 4 (Fig. 1) from mass spectral data presented in Figure 3 (scans 2, 3 and 4), fatty acid composition, and carbon number (Table 2). Mass spectral scan numbers refer to HPLC fraction numbers (Fig. 1). Mass spectral data for fractions 5 and 6 (Fig. 1) are presented in Figure 4 (scans 5 and 6). Mass ions at 551, 552, 575 and 576 $(M\text{-}RCO_2)^+$ show that the triacylglycerol 16:0 16:0 18:2 was present. Molecular ions (856, 884) for the other

TABLE 3

Identification of Triacylglycerol Components by Mass Spectrometry of HPLC Fractions

HPLC peak no.	Equivalent carbon no.	Peanut oil triglyceride			Mol wt.
1	42	18:2	18:2	18:2	879
2	44	18:1	18:2	18:2	881
3	44	16:0	18:2	18:2	854
4	46	18:1	18:1	18:2	882
5	46	16:0	18:1	18:2	856
6	46	16:0	16:0	18:2	830
	46	18:1	18:1	18:1	884
7	48	16:0	18:1	18:1	858
	48	16:0	16:0	18:1	832
	48	18:0	18:1	18:2	884
	48	18:1	18:2	20:1	910
	50	18:1	18:1	20:1	912
8	48	16:0	18:0	18:2	858
	50	18:0	18:1	18:1	886
	50	18:0	18:0	18:1	860
9	50	18:1	18:2	20:0	912
	52	18:1	18:1	20:0	914
	52	16:0	18:2	22:0	914
	54	16:0	18:1	22:0	916
10	52	18:1	18:2	22:0	940
	54	18:1	18:1	22:0	942
	56	18:1	18:2	24:0	970
	58	18:1	18:1	24:0	972

two triacylglycerols identified in these fractions are present. Multiple scans of fraction 7 (Fig. 4) revealed the presence of three triacylglycerols based on molecular ions 858, 884 and 912. Two additional triacylglycerols were identified in fraction 7 (Fig. 4) based on mass ions at 601, 603, 629, 631, 551, 552 and 577 $(M\text{-}RCO_2)^+$. Fatty acid composition of fraction 7 (Table 2) also indicates the presence of triacylglycerols containing a C-20:1 acyl moiety. Fraction 8 (Fig. 1) was composed of three triacylglycerols with the following molecular species having the highest concentration: 18:0 18:1 18:1 (molecular ion 886, scan 8, Fig. 5). Identification of the other two triacylglycerols was based on the transition ions $(M\text{-}18)^+$ (16) and the $(M\text{-}RCO_2)^+$ ions. Fatty acid compositions of fractions 9 and 10 (Table 2) show that all the fatty acid acyl moieties found in peanut oil are present in these two fractions. Therefore, triacylglycerols with combinations of these acyl moieties are present. Examination of the mass spectral information (scan 9 and 10, Fig. 5) confirms the presence of the remaining triacylglycerols.

A summation of the molecular species of triacylglycerols characterized is shown in Table 3. HPLC peaks on the high resolution chromatogram (Fig. 2) are labeled with the abbreviated form of the triacylglycerol.

As a result of this study, 23 triacylglycerols have been characterized. To our knowledge the separation in Figure 2 represents the highest resolution of peanut oil triacylglycerols reported (2,9), and the mass spectral data and GLC data presented have resulted in the most complete characterization of the major peanut oil triacylglycerols reported thus far by this methodology.

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