Comparative Study of the Extraction and Measurement of Cottonseed Free Fatty Acids

Peter J. Wan* and Michael K. Dowd

SRRC, ARS, USDA, New Orleans, Louisana 70124

ABSTRACT: Crude oil was extracted from cottonseed by three different methods to study the influence of extraction technique on the free fatty acid (FFA) concentration. Extraction procedures that recovered more oil had higher levels of FFA. In addition, the highest concentration of FFA was found in oil recovered by Soxhlet reextraction of a meal initially defatted by a room-temperature extraction process. The FFA concentrations of oils recovered by Soxhlet extraction were highly correlated with the FFA concentration of oils recovered by the other extraction methods studied ($R^2 > 0.96$). Titration of oil and gas chromatography of silylated oil were compared as methods to determine FFA concentration. The methods compared well ($R^2 = 0.998$) with the titration method, giving ~5% higher values for FFA than the chromatography method. Half of this difference appeared to be due to the oleic acid approximation used in the titration approach. The other half of the difference is likely due to the detection of other acidic components in crude oil.

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In the cottonseed processing industry, the concentration of free fatty acids (FFA) in seed directly affects seed grading and pricing, and the FFA concentration in extracted oil dictates the amount of caustic soda used during refining. Consequently, accurate and reliable methods are needed for measuring FFA levels in both seed and crude oil. In addition, accurate methods are needed by agriculturists working to improve cottonseed varieties as well as to improve harvesting and handling procedures.

Determination of FFA concentration in oilseeds is a two-step process. Initially, fatty materials, which include FFA, glycerides, phospholipids, oil-soluble pigments and other lipophilic substances, are extracted from the seed matrix. Numerous physical and chemical extraction methods are available for this purpose. Following extraction, FFA are generally measured by titration in an appropriate mixture of solvents.

In the Trading Rules of the National Cottonseed Products Association, AOCS Official Method Aa 6-38 is specified for determining the FFA concentration in cottonseed (1). This method recovers oil by room-temperature leaching of ground cottonseed meats with a Butt-type extraction tube and measures the FFA by titrating the oil in an isopropanol–hexane solvent mixture with sodium hydroxide (NaOH). Recent work indicates that the Aa 6-38 method (2) yields less oil than a 4-h Soxhlet-type extraction (3), and the oil recovered by the Aa 6-38 method has a lower FFA concentration than oil recovered by a 4-h Soxhlet extraction (3,4). These results indicate that FFA are extracted disproportionately by different oil recovery methods, and the determination of seed FFA is problematic in that the measured value is very sensitive to the method used for recovering oil. Consequently, the Aa 6-38 method may not provide a good estimate of the inherent FFA concentration in cottonseed.

Titration is the most commonly used method for determining FFA levels in cottonseed oils. While this method is generally considered reliable, the procedure is nonspecific, the determination assumes an average fatty acid molecular weight equal to that of oleic acid in converting from a molar to a mass basis, and no information is provided on the distribution of the individual fatty acids. In addition, dark pigments present in crude cottonseed oil (gossypol, etc.) can make the titration end point difficult to determine. A possible alternative is to use chromatography to separate and quantify the individual FFA. This approach has been successfully used to measure soap fatty acids in vegetable oil soapstocks (5,6).

This work was undertaken to further study the effect of extraction method on the concentration of FFA in crude cottonseed oil and to compare FFA measurements made by titration and by gas chromatography. Crude oil samples were obtained by hydraulic pressing of whole white cottonseed, by room-temperature leaching of ground cottonseed meats [AOCS Aa 6-38 (2)], and by Soxhlet extraction of ground cottonseed meats [AOCS Aa 4-38 (2)]. In addition, the residue from the room-temperature solvent extraction was reextracted by the Soxhlet method to provide an additional oil sample. This sample is indicative of the fatty material left behind by the current AOCS Aa 6-38 method. FFA concentration was measured by titration, as recommended by AOCS Aa 6-38 (2), and by a mild derivatization procedure followed by gas chromatography.

MATERIALS AND METHODS

Sample preparation. Twelve cottonseed samples were provided from a commercial oil mill. Because of the difficult harvesting

*To whom correspondence should be addressed at SRRC, ARS, USDA, 1100 Robert E. Lee Blvd., New Orleans, LA 70124.
E-mail: pwan@nola.srrc.usda.gov

conditions during the 1997–98 crushing season, the samples were known to have varying amounts of FFA. For the solvent-based recovery methods, the seeds were cracked in a 1-L Waring blender operated at full speed or in a pulsating manner until the majority of seeds were dehulled. Meats were recovered with a #7 mesh sieve and were ground in a Model G-3 Bunn Coffee Grinder (Springfield, IL) operated at the "drip" setting. The meats were ground to pass through a #14 sieve.

Oil extraction. For pressing, ~80 g of whole white (fuzzy) cottonseed was placed in a closed-bottom cylinder (1.5″ diameter or 3.81 cm) with an oil drainage tube located near the cylinder bottom. A plunger was compressed into the cylinder slowly until a final pressure of 5,000 psi or 34.5 MPa was reached. Room-temperature leaching was conducted as described in AOCS Aa 6-38 (2), using petroleum ether on 40–50 g samples of ground cottonseed meats. Soxhlet extractions were conducted in a manner similar to the extraction process described in AOCS Aa 4-38 (2), except that a Soxhlet extraction tube was used in place of the recommended Butt tube. The Soxhlet procedure was conducted for 4 h with petroleum ether. The Soxhlet procedure was also used to recover oil from the defatted meal previously extracted by the room-temperature leaching process. Crude oil was recovered from miscella by evaporating the petroleum ether with a rotary evaporator operated for 1 h with a maximal still temperature of 60°C and a final absolute pressure of 3 in. of Hg.

FFA determination. For most of the oils, 4–5 g samples were dispersed in isopropanol (75 mL) and hexane (15 mL) followed by titration against 0.25 N NaOH, as described in AOCS Aa 6-38 (2). Because the amount of oil recovered by the Soxhlet reextraction of the defatted residue was small, the amount of oil used in the FFA determination was reduced and the solution was titrated against 0.1 N NaOH.

For analysis by gas chromatography, the oils were silylated to improve the volatility of the FFA and glyceride components. Silylation chemicals were purchased from Pierce (Rockford, IL). Crude oil (~100 mg) was weighed into a septum vial, followed by pyridine (1 mL) containing a known amount of internal standard (cholesterol methyl ether), hexamethyldisilazane (1 mL), and trifluoroacetic acid (100 µL). Solutions were capped and heated at 60°C for 45 min before chromatography. The trimethylsilylated FFA (TMS-FFA) were separated using a J&W Scientific (Folsom, CA) DB-5ht column (15 m × 0.25 mm i.d. × 0.1-mm film thickness) with a split injector (340°C, 1:50), flame-ionization detector (340°C), and helium carrier gas (~1 mL/min). The column temperature was initially held at 100°C for 3 min, then increased to 150°C at 10°C/min, then increased to 250°C at 5°C/min, then increased to 360°C at 10°C/min, and held at 360°C for 10 min. The final temperature was sufficient to elute cottonseed triglycerides.

Fatty acid peaks were identified by comparing elution times with the times for known standards of silylated fatty acids. Fatty acid samples were purchased from Sigma-Aldrich (St. Louis, MO) and derivatized as described above. FFA concentrations were determined by internal standardization (7). Response factors were similar for the individual fatty acids—varying in range by about 5%. All samples were analyzed in duplicate, except for two of the twice-extracted samples, which did not yield sufficient oil for duplicate determinations.

RESULTS AND DISCUSSION

Effect of extraction methods on FFA. The seed samples used in this study included a range of FFA concentrations from <2.0 to >40% (Table 1). For all of the seed samples, the measured concentration of FFA in the extracted oil greatly depended on the method used to recover the oil (Table 1). As found previously (3,4), the concentration of FFA in oil extracted by room-temperature leaching was consistently lower than the concentration of FFA in oil recovered by Soxhlet extraction. In addition, the oil recovered by pressing whole seed was markedly lower

TABLE 1
FFA Concentration (wt%) of Extracted Cottonseed Crude Oils Measured by Titration and Gas Chromatography[a]

Extraction method:	Hydraulic pressing			Room-temperature solvent extraction			Soxhlet solvent extraction			Soxhlet solvent extraction of pre-extracted meal[b]		
Sample no.	Titration	GC	GC (oleic)[c]	Titration	GC	GC (oleic)	Titration	GC	GC (oleic)	Titration	GC	GC (oleic)
1	22.6	21.0	21.5	38.4	37.2	38.4	41.4	39.1	40.5	44.5	48.0	50.1
2	6.8	6.05	6.21	8.1	8.19	8.45	9.5	9.16	9.47	13.1	11.90	12.36
3	1.4	ND[d]	ND[d]	7.2	7.48	7.72	9.2	8.24	8.51	13.0	13.0	13.4
4	3.3	3.48	3.57	6.0	6.06	6.26	7.8	7.35	7.6	10.6	9.11	9.49
5	20.5	19.0	19.4	34.5	33.7	34.7	38.0	35.0	36.1	43.9	42.3	44.0
6	8.5	7.6	7.8	13.6	12.8	13.2	16.2	14.1	14.6	23.6	19.5	20.2
7	2.2	1.77	1.82	4.1	4.25	4.40	4.4	5.59	5.78	5.6	10.2	10.6
8	4.0	3.62	3.72	7.1	6.75	6.96	8.4	7.57	7.82	10.0	9.75	10.1
9	5.9	5.31	5.45	8.6	7.64	7.88	9.1	8.29	8.57	12.6	11.3	11.8
10	1.9	1.59	1.63	2.9	2.63	2.72	3.8	3.07	3.18	6.9	5.65	5.87
11	2.5	2.08	2.14	5.7	5.33	5.50	6.5	6.91	7.14	8.7	8.42	8.75
12	6.5	5.88	6.01	8.4	7.68	7.92	8.8	9.11	9.41	11.6	11.1	11.5

[a]FFA, free fatty acid; GC, gas chromatography.
[b]Reextraction of the meal residual from the room-temperature extraction method.
[c]Values "adjusted" to an oleic acid weight basis.
[d]ND = not determined; sample inadvertently destroyed.

in FFA than either of the solvent-based recovery methods. Oil recovered by Soxhlet reextraction of the room-temperature-leached ground meats had consistently higher levels of FFA than the oils extracted by the other methods (Table 1).

Based on a linear model ($y = ax + b$), the FFA concentration of Soxhlet-extracted oil was strongly correlated with both the FFA concentration of oil recovered by hydraulic pressing [coefficient of determination (R^2) = 0.961] and the FFA concentration of oil recovered by room-temperature leaching (R^2 = 0.998) (Fig. 1). Linear regression statistics are given in Table 2. The FFA concentration of the Soxhlet-extracted oil was also highly correlated with the FFA concentration of the oil recovered by the Soxhlet reextraction of meats initially defatted by leaching (R^2 = 0.987). The previously reported (3) regression slope between the FFA concentration of Soxhlet-extracted oil and room-temperature-leached oil was similar to the value obtained in this work. Individual *F*-tests for homogeneity of slopes and homogeneity of intercepts between the data reported in Reference 3 and the data reported in this work indicated that there was no significant difference in the two sets of regression parameters (probability value, $P > 0.05$). Because the regression results for the two sets of data were statistically the same, a statistical model based on the combined data set is believed to be best for estimating Soxhlet-based FFA values from room-temperature-leached oil samples (Table 2).

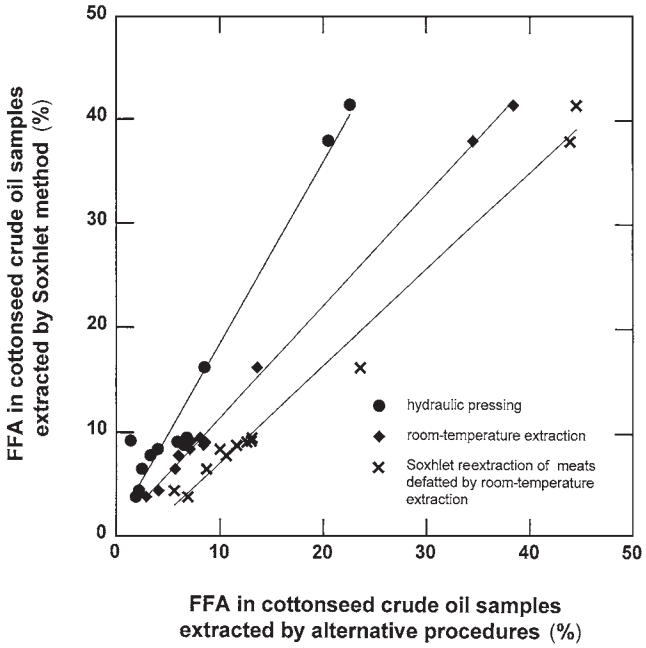

FIG. 1. Correlation between free fatty acid (FFA) concentration of crude oil recovered by 4-h Soxhlet extraction and the FFA concentration of crude oil recovered by hydraulic pressing, solvent leaching, and 4-h Soxhlet extraction of previously leached material. FFA concentrations were determined by titration.

The results confirm that FFA are not extracted at the same rate as the glyceride components and that FFA levels are higher in cottonseed oils recovered from extraction processes that more thoroughly extract oil. Consequently, accurate measurement of the FFA concentration in cottonseed requires complete extraction of the oil.

However, because the FFA concentrations of oils extracted by different techniques tend to be strongly correlated, seed FFA levels can be estimated by combining a quick oil recovery method with a statistical correlation between the FFA concentrations of oil recovered by the quick method and oil recovered by a more thorough procedure. It should also be possible to estimate the FFA content of "extractable" oil by combining a quick extraction approach with a correlation incorporating an extraction procedure that mimics industrial operations. This methodology may be particularly useful where a rapid determination of the seed FFA content is desired.

Compared with exhaustive extraction procedures that use multiple solvent extraction and milling steps, Soxhlet extraction (4-h) recovers ~95% of the extractable fatty material from finely ground cottonseed meats (4). Dehulled cottonseed kernels contain 32–34% lipid material, and defatted meal typically contains ~0.8–1.2% residual fat, which includes the addition of ~5% of the initial kernel weight in hulls (D.E. Britton, Mid-Continental Laboratories, personal communication). By material balance, lipid recoveries of ~97% are achieved during industrial operations. Of the methods considered in this study, the Soxhlet method appears to provide a comparable yield of crude oil and, consequently, is also likely the best estimate of the FFA concentration in "extractable" oil. The Aa 6-38 method appears to underestimate both the inherent seed FFA concentration and the FFA concentration in the "extractable" oil.

Measurement of FFA concentration by titration and gas chromatographic method. Good agreement was found between FFA concentration measured by titration and by gas chromatography (Fig. 2). Generally, the titration procedure yielded slightly higher results as indicated by the slope of the linear ($y = ax$) regression model between the two methods.

TABLE 2
Correlation Statistics Between Measured FFA Concentration of Crude Oils Extracted by Different Procedures and the FFA Concentration of Oils Extracted by a 4-h Soxhlet Extraction Method (dependent variable)[a]

Extraction method (independent variable)	Slope	Intercept	R^2	Reference
Hydraulic pressing	1.743	–1.083	0.961	This work
Room-temperature leaching (AOCS Aa 6-38)	1.073	0.664	0.998	This work
	1.124	0.178	0.997	3
	1.084	0.431	0.998	Combined data
Soxhlet extraction of AOCS Aa 6-38 residue	0.930	–2.220	0.987	This work

[a]By titration. See Table 1 for abbreviation.

For the complete set of data, the slope was 1.032 (R^2 = 0.994). However, because of the limited amount of oil recovered by the Soxhlet reextraction of the defatted meal samples, smaller amounts of oil were used for the titration measurements, and two of the twice-extracted samples appeared to be statistical anomalies (Fig. 2). If these two points are excluded from the analysis, the regression slope increases to 1.059 and the coefficient of determination improves to 0.998. Essentially the same result is found if all of the twice-extracted samples are excluded from the analysis (slope = 1.056, R^2 = 0.998). Taking the slope to be ~1.05 suggests that the titration method on average overestimates FFA by ~5%. This difference is likely due to the measurement of other acidic components within the crude oil and the arbitrary use of the molecular mass of oleic acid for estimating the concentration of FFA on mass basis from the determined molar acidity.

Other components that may interfere with the titration of FFA in cottonseed oil include gossypol and small molecular weight organic and inorganic acids. Gossypol is a polyphenolic pigment and is a significant component of crude cottonseed oil (0.2–0.8%). Because gossypol has six phenolic hydroxyl groups, the compound behaves as a weak acid, and at least one cottonseed processor has incorporated a factor to account for gossypol in estimating FFA levels in crude oil (Watkins, L., Texas A & M University, personal communication). A number of low molecular weight acids have also been identified in oil refining by-products (5,6,8). Trace levels of phosphoric acid were apparent in the chromatograms of this work, and this acid has been reported in soapstock (5,6) and acid water (8). Although phosphoric acid is sometimes used during oil refining to facilitate or enhance phospholipid removal, this processing additive is not believed to have influenced the results in References 5, 6, and 8 because much higher levels would have been expected had this occurred. Lactic, glyceric, and gluconic acids have also been identified in wastewater from the acidification of cottonseed soapstock (8). The presence of these acids in refining by-products indicates that they are also likely present in crude oil and contribute to oil acidity.

Because palmitic acid is a significant component among the FFA of crude cottonseed oil (~25%, see below), the use of oleic acid as a molar mass reference tends to elevate the estimation of FFA concentration. The magnitude of this effect can be approximated as the difference in the molecular masses of palmitic and oleic acids divided by the molecular mass of palmitic acid multiplied by the percentage of palmitic acid within the FFA fraction. For the cottonseed samples, the effect is ~2.5%. This is about half of the difference between the titration and gas chromatographic methods noted above. While it is not possible to correct the titration values without prior knowledge of the FFA distribution, it is possible to "adjust" the gas chromatographic values to mimic this effect. This was achieved by converting the masses of each fatty acid to a molar basis and recalculating the mass percentages assuming all of the fatty acids have the molecular mass of oleic acid (Table 1). Correlating FFA measured by titration against gas chromatography with the oleic acid "adjustment" lowered the regression slope to 1.025 (R^2 = 0.998). The change in the regression slope also suggests that approximately half of the

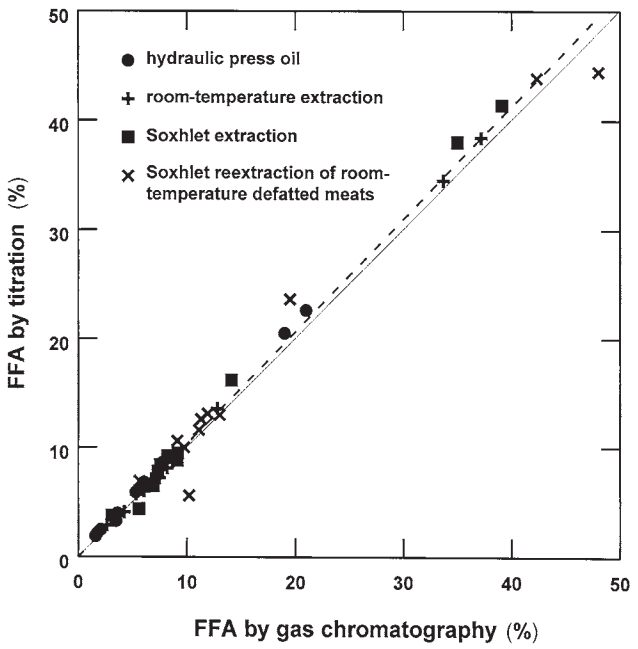

FIG. 2. Correlation between FFA concentration determined by gas chromatography and FFA concentration determined by titration. See Figure 1 for abbreviation. Dashed line: best fit of the experimental data; solid line, perfect fit.

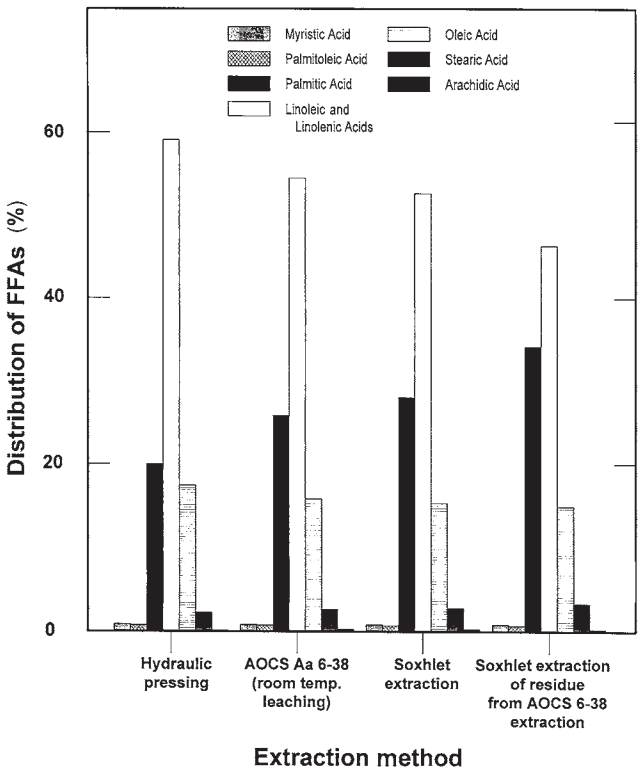

FIG. 3. Average distribution of FFA in oil samples extracted by different methods. Averages were based on 11 samples. One sample was excluded in the analysis because only partial data were available (see Table 1). See Figure 1 for abbreviation.

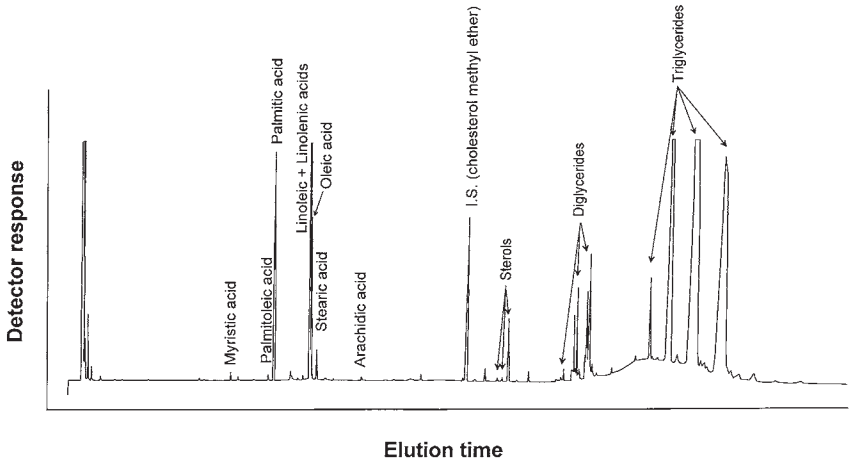

FIG. 4. Gas chromatogram of silylated crude cottonseed oil using the chromatographic conditions described in the text. I.S., internal standard.

difference between the two measurements occurs because of the assumption of an oleic acid basis for the titration method.

In a previous report (3), the fraction of saturated fatty acids in the total fatty acid profile was found to be higher for oil recovered by Soxhlet extraction than for oil recovered by room-temperature leaching. It was not clear, however, if the distribution differences were due to differences in the glyceride components, FFA components, or a combination of the two. One advantage of the gas chromatographic method for FFA analysis is that it also yields information about the distribution of FFA. In this study, the percentage of saturated fatty acids in the FFA distribution increased in the order: hydraulic pressing < room temperature extraction < Soxhlet extraction (Fig. 3). The highest levels of saturated FFA were found in the oil recovered from the residue of the room-temperature extracted material. The distribution changes appear to indicate that saturated FFA are more difficult to extract than unsaturated FFA, and changes in the FFA distributions account for at least a part of the total fatty acid differences seen earlier.

Gas chromatographic measurement of FFA in an oil-containing material is to some degree problematic. The acid components of vegetable oil can be derivatized to improve their volatility for thermal analysis without significantly degrading the glyceride components. Ideally, a gas chromatographic stationary phase is needed that will separate the derivatized fatty acids and elute the less volatile glyceride components. New high-temperature stationary phases elute triglycerides, but these phases tend to be nonpolar and do not yield an ideal separation of all the TMS-unsaturated fatty acids found in vegetable oils.

On the 5% phenyl–95% methyl stationary phase used in this work, TMS-linoleic and TMS-linolenic acids coeluted. In addition, baseline-to-baseline separation of TMS-oleic and TMS-linoleic acids could not be achieved with the sample loading needed to quantify the minor fatty acids (Fig. 4). Though not ideal, these chromatographic problems were manageable for the purpose of estimating the FFA content in crude oils. Response factors for the unresolved TMS-linoleic and TMS-linolenic acids were essentially the same at the conditions used. Hence, even though the ratio of these individual components could not be determined, the sum of the two acids was not significantly in error. Also there was sufficient resolution of the TMS-oleic acid and TMS-linoleic/linolenic acids that peak partitioning did yield realistic and reproducible ratios for these components.

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