Analysis of the Monoenoic Fatty Acid Distribution in Hydrogenated Vegetable Oils by Silver-Ion High-Performance Liquid Chromatography

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ABSTRACT: A silver-ion high-performance liquid chromatography column (hexane/acetonitrile as solvent, ultraviolet detection) was used to analyze the fatty acid distribution (as fatty acid methyl esters) of a representative sample of hydrogenated oil. Fractions containing *cis*- and *trans*-18:1 isomers were readily separated. The positional fatty acid isomers were separated by rechromatographing these fractions. The elution order and percent compositions were compared with results obtained by gas chromatography. Of the Δ8 to Δ14 *trans*-18:1 isomers, only the Δ8 and Δ9 pair could not be separated. The Δ8 and Δ9 *cis*-18:1 pair also could not be separated, and the Δ10 isomer was poorly separated from this pair. Area percents were comparable to results obtained by gas chromatography.
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KEY WORDS: *cis* Isomers, fats and oils, fatty acids and esters, hydrogenation, isomers, liquid chromatography, silver and silver compounds, *trans* isomers, unsaturated fatty acids and esters.

Partial hydrogenation of vegetable oils produces both configurational (*cis* vs. *trans*) and positional (double-bond location; may vary from the Δ8 to the Δ15 position on the carbon chain) isomers (1–3). Due to the large number of isomers generated during the hydrogenation process, attempts to determine the monoenoic (18:1) fatty acid isomer distribution of the vegetable oil by analysis of the triacylglycerols (TAG) is difficult (4,5). If the TAG are first converted to fatty acid methyl esters (FAME), gas chromatography (GC) and high-performance liquid chromatography (HPLC) can then be used to determine the *cis* and *trans* 18:1 FAME distribution (2,3,6,7). Determination of the positional isomer distribution by GC generally requires 50- or 100-m highly polar, fused silica capillary columns such as SP2560 or CP Sil-88 (8–10). Despite the tremendous separation capabilities of these GC columns, some *cis/trans* 18:1 isomer overlap still occurs (*trans*-14-18:1 and *cis*-9-18:1, *trans*-13-18:1 and *cis*-8-18:1, for example). This problem is reflected in the difficulty of devising a standard GC method for the analysis of partially hydrogenated oil FAME (11). To obtain a more complete FAME profile, the *cis*- and *trans*-18:1 fractions are often separated by silver nitrate thin-layer chromatography (TLC) before the positional isomers are characterized by GC and Fourier transform infrared (3), or by such technologies as microozonization (12) or derivatization/mass spectrophotometry (13).

Silver-ion HPLC (Ag-HPLC) has been used to separate (as the FAME) a wide variety of lipid structures by the number of and/or configuration (*cis* vs. *trans*) of the double bonds (14–19), but the fractionation of TAG configurational, as well as TAG and FAME positional isomers, by Ag-HPLC has been less successful (15,18). Recently, Ag-HPLC on a commercially available HPLC column with a solvent system of acetonitrile (ACN) in hexane and ultraviolet (UV) detection (14) was used to separate the *cis* and *trans* FAME isomers of methyl oleate, methyl linoleate, methyl linolenate, and 15 of the 16 *cis/trans* isomers of methyl arachidonate. In this paper, we describe the extension of Ag-HPLC to the fractionation and characterization of partially hydrogenated vegetable oil FAME.

EXPERIMENTAL PROCEDURES

Materials and reagents. Hexane (Allied Fisher Scientific, Orangeburg, NY) and ACN (E. Merck, Darmstadt, Germany) were HPLC-grade, and all solvents were used as received. Isooctane was ACS-grade. A sample of partially hydrogenated vegetable oil, containing a range of positional and geometric 18:1 fatty acid isomers, was obtained from a commercial source. The sample was converted to methyl esters with HCl/methanol (4) and eluted with petroleum ether through a silica gel Sep-Pak (Waters Associates, Milford, MA). After solvent removal, the samples were dissolved in isooctane (*ca.* 10 mg/mL).

HPLC. HPLC analyses were performed with a Spectra Physics P2000 isocratic solvent delivery system (Spectra Physics Analytical, Freemont, CA), a Rheodyne 7125 injector (Rheodyne, Inc., Cotati, CA) with a 20-μL injection loop,

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and an ISCO V4 Absorbance Detector (Isco, Inc., Lincoln, NE) set at 206 nm. Refractive index (RI; Model 403; Waters Associates) and flame-ionization detectors (FID) (Tracor Model 945; Tremetrics, Austin, TX) were also used. The ChromSpher lipids column (4.6 mm i.d. × 250 mm stainless steel, 5μ particle size, commercially available in silver ion form) was purchased from Chrompack International (Middelburg, The Netherlands). Solvent flow was standardized at 1.0 mL/min.

Analyses of HPLC fractions. FAME fractions were collected and analyzed by GC. Routine analysis of fractions was accomplished with a Varian 3400 GC (Varian Instruments, Palo Alto, CA) equipped with a 30 m × 0.32 mm SP2380 (Supelco, Inc., Bellefonte, PA) capillary column and FID, and He was used as carrier gas. Operating conditions were: injector, 240°C; split ratio, 100:1; oven temperature programmed from 155 to 220°C at 2°C per min with an initial hold of 15 min; detector, 280°C. Positional isomers were analyzed by a Varian 3400 GC equipped with a 100 m × 0.25 mm SP 2560 fused-silica capillary column (Supelco, Inc.), and connected to an FID; He was used as carrier gas. GC operating conditions: injector and detector temperatures, 235°C; split ratio, 100:1; oven temperature, 175°C for 25 min, then programmed to 235°C at 10°C per min. Methyl ester peaks were identified and quantitated with a standard FAME mixture of known composition (Cat. No. 4-5170; Supelco, Inc.). Most samples were run in triplicate; the positional 18:1 isomer analyses (UV detector, see above for experimental details) were repeated seven times. Detector signal output was monitored by computer, and the isomer percents were determined as described previously (20). Peak deconvolution (21) was used to increase the accuracy of the data.

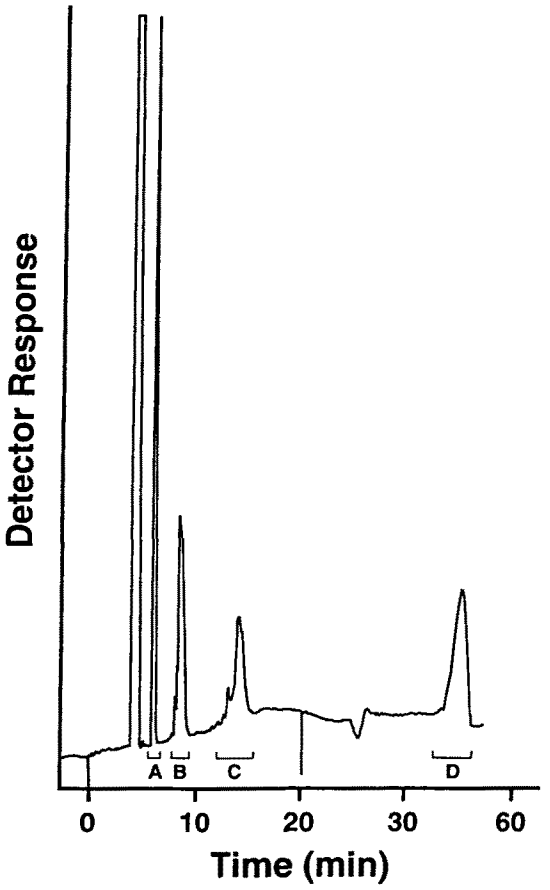

FIG. 1. Analysis of partially hydrogenated vegetable oil fatty acid methyl esters by silver-ion high-performance liquid chromatography. Sample size, 20 μg; flow rate, 1.0 mL/min 0.15% acetonitrile in hexane; RI detector. Fractions: A, saturates; B, *trans*-18:1; C, *cis*-18:1; D, 18:2.

RESULTS

A typical Ag-HPLC chromatogram of the FAME from a partially hydrogenated vegetable oil is presented in Figure 1; RI detection was used to allow comparison with percent compositions derived from GC analysis (Fig. 2). The *trans*- and *cis*-18:1 isomer patterns shown in Figure 2 (inserts A and B, respectively), were obtained by GC analysis (100-m SP2560) of fractions B and C (Fig. 1). The elution patterns (UV detection) of the *cis*- and *trans*-18:1 fractions are presented in Figure 3. Isocratic solvent conditions (0.08% ACN in hexane) were used to maximize 18:1 positional isomer separations, and 0.4% ACN in hexane was used to elute the polyunsaturated FAME. The percent composition data obtained by HPLC (UV, RI, and FID) and GC (100 m SP2560) analyses are compared in Table 1.

DISCUSSION

Reproducibility/quantitation of data. Quantitation of the HPLC and GC data are compared in Table 1. Good reproducibility (±4% of peak area or better) was achieved with Ag-HPLC (FID), but positional isomer resolution was reduced because a larger sample size was required for detection. Reproducibility of the Ag-HPLC (RI) system was 5–6% of peak area. Comparison of the results obtained by Ag-HPLC (FID) with GC–FID for the saturated, *trans*-18:1, *cis*-18:1 and 18:2 FAME varied from 1 to 10% of peak area. A similar relationship was observed with RI detection but was further complicated when the RI response for 18:2 was roughly twice that observed with Ag-HPLC (FID) or GC. Correction factors would be required for quantitation by Ag-HPLC (RI).

Optimized separations. The optimized separation of the *cis*- and *trans*-18:1 positional isomers is illustrated in Figure 3. Ag-HPLC was unable to separate the Δ8 and Δ9 18:1 positional isomer pairs (neither *cis* nor *trans*), and the Δ10 *cis*-18:1 isomer was often poorly separated from the *cis*-Δ8 and *cis*-Δ9 isomer pair. These results are consistent with the TLC and Ag-HPLC elution patterns noted by other investigators (18). The use of two ChromSpher lipids columns in series (FID detection) was investigated in an attempt to obtain improved 18:1 positional isomer separations, but it was only partially successful. Although the separation of *cis*-10-18:1 from the *cis*-Δ8/Δ9 FAME pair was improved, resolution of the Δ8 and Δ9 isomers was not.

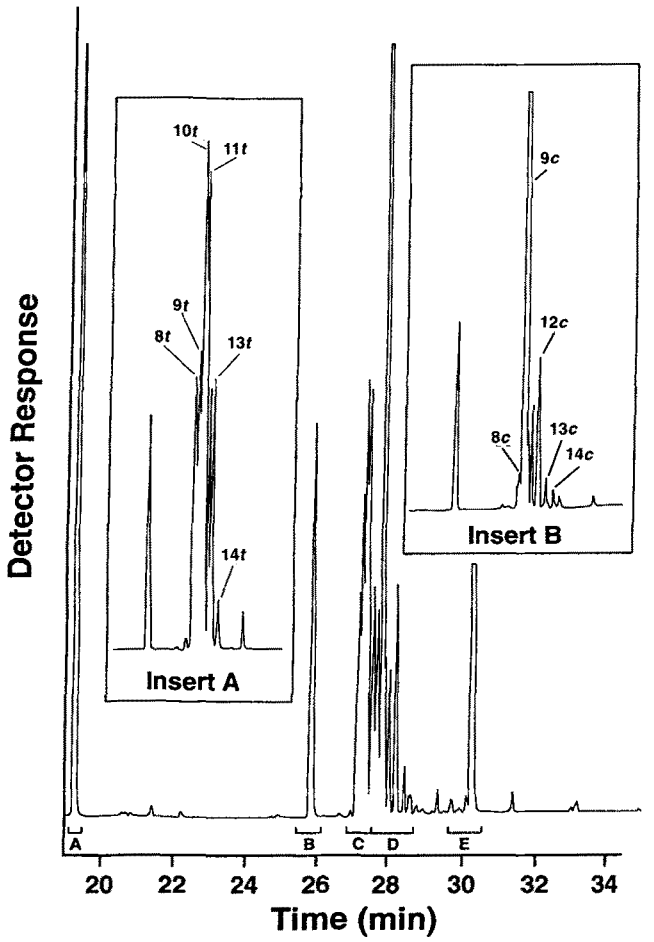

FIG. 2. Segment of gas chromatogram (100 m SP2560) of partially hydrogenated vegetable oil fatty acid methyl ester. Inserts A (*trans*-18:1 isomer distribution) and B (*cis*-18:1 isomer distribution) were obtained by gas chromatographic analysis of fractions C and D in Figure 1, respectively. Fractions: A, 16:0; B, 18:0; C, *trans*-18:1; D, *cis*-18:2; E, *cis/trans, trans/cis* and *cis/cis*-18:2.

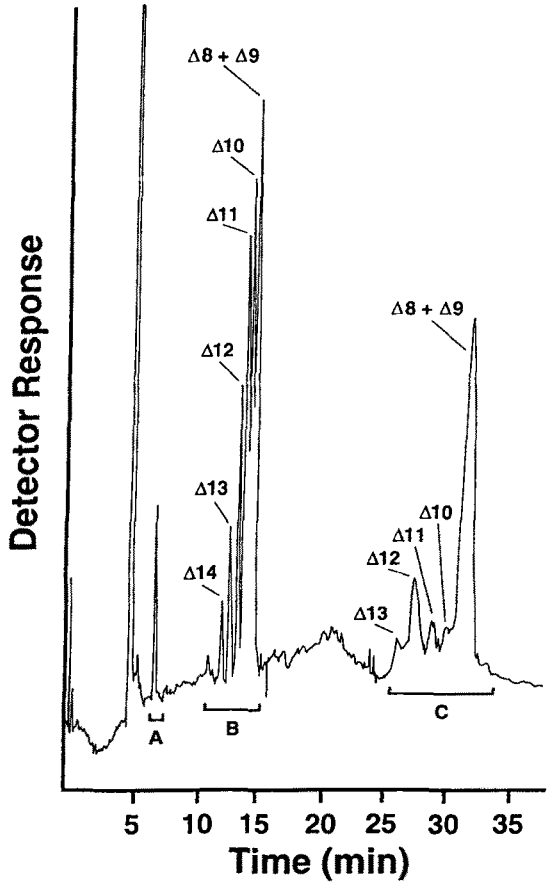

FIG. 3. Analysis of partially hydrogenated vegetable oil 18:1 fatty acid methyl ester positional isomers by silver-ion high-performance liquid chromatography. Sample size: 0.4 μg; flow rate, 1.0 mL/min 0.08% acetonitrile in hexane; ultraviolet detector at 206 nm. Fractions: A, saturates; B, *trans*-18:1; C, *cis*-18:1.

Optimum *cis*-10-18:1 resolution was achieved by using dual columns and, more importantly, by decreasing sample size to 0.5 μg or less. A UV detector was required for the positional 18:1 FAME isomer analyses due to its greater sensitivity when compared with RI or FID. Based on first principles, we assumed that UV responses for monoenoic FAME would be the same regardless of double-bond location on the carbon chain (Δ8 to the Δ15 position). The data in Table 1 indicate this assumption to be reasonable. The elution orders of the 18:1 positional isomers (*cis* and *trans*) are the reverse of those obtained by capillary GC (SP2560; Fig. 2).

Sample loading. The low sample capacity of the Chrom-Spher lipids column required sample size to be limited to 0.5 μg or less for optimum isomer separations. At these low sample loadings, Ag-HPLC (UV) data agreed well with results obtained by GC–FID for *trans*-Δ8/Δ9 through Δ12 (within 10–12% of peak area), but less so for the Δ13 and Δ14 *trans* isomers (10 to >50% of peak area). The area percents from the HPLC of the *cis* positional isomers equated with the GC–FID data to within 10–20% of peak area. The Δ14 and Δ15 *cis* isomers could not be distinguished from baseline noise in the HPLC trace at these low sample loadings. The sample sizes utilized in our study may be contrasted with the 0.1–0.5 mg employed by other researchers (17–19) on these types of HPLC columns. Significant resolution losses occurred when injected sample sizes were increased to even 0.1 or 0.2 mg.

Limitations/applications. The Ag-HPLC method has several advantages, including rapid (15–20 min run-time) analysis and complete separation of *cis*- and *trans*-18:1 isomers (with no *cis/trans* overlap). The complete separation of *cis* and *trans* isomers can be used to provide quantitative data for positional isomers not separated by GC. However, Ag-HPLC is more limited than GC as a stand-alone method for the study of individual, positional FAME isomers. This technology is, therefore, a good alternative to silver-nitrate TLC for preliminary isomer fractionation before GC analysis and as a method for isolation of most of the 18:1 positional isomers.

TABLE 1
Comparison of Analytical Methodologies for Determination of Fatty Acid Methyl Ester (FAME) Isomer Distribution in Partially Hydrogenated Vegetable Oils

Method of analysis[a]	%Sat.[b]	*trans*-18:1 Isomers (%)[c] 14	13	12	11	19	9	8	Sum *trans*[d] (%)	*cis*-18:1 Isomers (%) 15	14	13	12	11	10	9	8	Sum *cis* (%)	*cis*-18:2 (%)
Ag-HPLC																			
UV detection		2.4	7.0	12.4	20.5	26.2		31.6[e]				2.7	10.3	7.9	5.5		72.8[e]		
±SD		0.2	0.3	0.4	0.8	1.2		1.3				0.8	1.4	1.5	0.8		2.2		
GC–FID	26.6	2.1	12.0	11.9	18.2	26.2	14.4	15.2	27.3	0.8	1.4	2.5	12.6	8.6	5.0	68.3	3.0	26.3	17.7
±SD	0.7	0.0	0.0	0.1	0.1	0.0	0.2	0.2	0.2	0.1	0.1	0.0	0.1	0.0	0.1	0.2	0.0	0.2	0.1
Ag-HPLC																			
RI detection	20.7								24.0									19.2	36.2
±SD	0.8								0.4									1.0	1.8
Ag-HPLC																			
FID	25.5								30.1									26.9	17.5
±SD	0.7								1.1									0.9	0.5

[a]Method used to determine FAME distribution. Ag-HPLC, silver-ion high-performance liquid chromatography; UV, ultraviolet; GC, gas chromatography; FID, flame-ionization detection; RI, refractive index; Sat., saturate FAME.
[b]Combined 10:0 through 20:0 FAME.
[c]Number denotes position of double bond.
[d]Total *trans*-18:1 in sample.
[e]8- and 9-18:1 Isomers co-eluted.

REFERENCES

1. Slover, H.T., R.H. Thompson, C.S. Davis and G.V. Merola, *J. Am. Oil Chem. Soc. 62*:775 (1985).
2. Mossoba, M.M., R.E. McDonald, D.J. Armstrong and S.W. Page, *J. Agric. Food Chem. 39*:695 (1991).
3. Ulberth, F., and M. Henninger, *J. Am. Oil Chem. Soc. 69*:829 (1992).
4. Evershed, R.P., in *Lipid Analysis, A Practical Approach*, edited by R.J. Hamilton, and S. Hamilton, Oxford University Press, New York, 1993, pp. 130–133.
5. Christie, W.W., *Lipid Analysis*, 2nd edn., Pergamon Press, 1982, p. 53.
6. Ratnayake, W.M.N., R. Hollywood, E. O'Grady and J.L. Beare-Rogers, *J. Am. Oil Chem. Soc. 67*:804 (1990).
7. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th edn., edited by D. Firestone, American Oil Chemists' Society, Champaign, 1990, Method Ce 1c-89.
8. Ratnayake, W.M.N., and G. Pelletier, *J. Am. Oil Chem. Soc. 69*:95 (1992).
9. Wolff, R.L., *J. Chromatogr. Sci. 30*:17 (1992).
10. Wolff, R.L., *J. Am. Oil Chem. Soc. 69*:106 (1992).
11. Ratnayake, W.M.N., *Ibid. 69*:192 (1992).
12. Roehm, J.N., and O.S. Privett, *J. Lipid Res. 10*:245 (1969).
13. Christie, W.W., *Advances in Lipid Methodology—One*, The Oily Press, Scotland, 1992, p. 89.
14. Adlof, R.O., *J. Chromatogr. A 659*:95 (1994).
15. Christie, W.W., and G.H. McG. Breckenridge, *Ibid. 469*:261 (1989).
16. Christie, W.W., *J. High Resolut. Chromatogr. Commun. 10*:148 (1987).
17. Christie, W.W., E.Y. Brechany and K. Stefanov, *Chem. Phys. Lipids 46*:127 (1988).
18. Nikolova-Damyanova, B., B.G. Herslof and W.W. Christie, *Ibid. 609*:133 (1992).
19. Toschi, T.G., P. Capella, C. Holt and W.W. Christie, *J. Sci. Food Agric. 61*:261 (1993).
20. Butterfield, R.O., W.K. Rohwedder, E.D. Bitner, J.O Ernst, D.J. Wolf and H.J. Dutton, *Prog. Lip. Res. 17*:93 (1978).
21. Beyers, E.C., and E.A. Emken, *Biochim. Biophys. Acta 1082*:275 (1991).

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