Gas Chromatographic/Matrix Isolation/Fourier Transform Infrared Spectroscopic Determination of *trans*-Monounsaturated and Saturated Fatty Acid Methyl Esters in Partially Hydrogenated Menhaden Oil

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Elaidic acid (*t*-18:1) has been the focus of a literature debate about its effect on blood cholesterol levels. A method is presented that can accurately quantify the level of *t*-18:1, a single geometric isomer, in hydrogenated menhaden oil. Fatty acid methyl esters (FAMEs) were determined by Fourier transform infrared spectroscopy. The *t*-18:1 FAME and related *trans*-monounsaturated and saturated compounds were quantitated by linear regression analysis from the intensities of the 971- and 1121-cm^{-1} infrared bands, respectively. The 971-cm^{-1} band is produced for *trans* geometric isomers only; thus, interference from *cis* isomers, due to GC peak overlap, is eliminated. At an iodine value of 78, the *t*-18:1 composition of hydrogenated menhaden oil was 2.6% by weight. The use of internal standards was essential for quantitation. Satisfactory repeatability was achieved for quantitation of FAMEs from duplicate high-performance liquid chromatographic fractions; the *t*-18:1 FAME weight percent varied by 7.4%.

INTRODUCTION

Polyunsaturated fatty acids (PUFA) in refined menhaden oil have as many as five [eicosapentaenoic acid (EPA), 22:5] or six [docosahexaenoic acid (DHA), 22:6] double bonds, which increase their susceptibility to oxidation and flavor deterioration. To stabilize the oil, the levels of PUFA are reduced or eliminated through partial hydrogenation. This selective process converts the fatty acid double-bond configuration from *cis* to *trans* and produces a fat with the desired plasticity.

Although the nutritional value of fatty acids has been extensively investigated, the role of *trans* geometric isomers of fatty acids in partially hydrogenated fish oils has yet to be addressed. Data by Mensink and Katan (1990) indicate that the monounsaturated *trans*-18:1 (elaidic acid) geometric isomer raises low-density lipoprotein (LDL) cholesterol and also lowers high-density lipoprotein (HDL) cholesterol when compared to a *cis*-18:1 (oleic acid) control. As a result of this finding, Grundy (1990) suggested that, unlike other unsaturated fatty acids (e.g., oleic and linoleic), the *trans*-monounsaturates should be labeled "cholesterol-raising" compounds. However, most researchers agree that further study should be carried out first (AOCS, 1990). Recently, Willett et al. (1993) reported that consumption of partially hydrogenated vegetable oils, a major source of *trans* isomers of fatty acids, may contribute to the occurrence of coronary heart disease.

Because the nutritional value of unsaturated fatty acids in hydrogenated oils may depend strongly on their double-bond configuration, a method is needed to quantify and confirm the identity of the *trans*-monounsaturated C_{18} fatty acid methyl ester (FAME) and its C_{16}, C_{20}, and C_{22} analogues, including saturated FAMEs, in hydrogenated menhaden oil. Such an analysis was carried out by using high-performance liquid chromatography (HPLC) and gas chromatography/matrix isolation/Fourier transform infrared (GC/MI/FT-IR) spectroscopy. GC/MI/FT-IR quantitation was based on the intensity of bands that are unique to *trans* (971 cm^{-1}) and saturated (1121 cm^{-1}) FAMEs, thus making the determination totally independent of any GC peak overlap. Problems of peak overlap in GC separations of hydrogenated edible oil FAME components are well documented (Ratnayake and Beare-Rogers, 1990). The quantitative method presented here is based on measurement of characteristic IR spectroscopic band intensity rather than GC peak area.

EXPERIMENTAL PROCEDURES

Materials. Refined menhaden oil was purchased from a local distributor. A nickel catalyst (20% Ni) was obtained from a commercial source.

Standard and Test Sample Preparation and Analysis. All solutions were prepared in isooctane. FAME standards were purchased from Alltech Associates (Deerfield, IL) and Nu-Check-Prep, Inc. (Elysian, MN). The test solutions were prepared from hydrogenated transesterified menhaden oil.

Hydrogenation and Esterification. Menhaden oil was hydrogenated in a 1-gal pressure vessel reactor (Autoclave Engineers, Erie, PA) at 1000 rpm and 207-kPa H_2 pressure. The nickel catalyst was used at a level of 0.2% [2 g:1000 g (catalyst:oil)] for hydrogenation, which was carried out at 150 ● 10°C for 10 min to obtain an iodine value of 78, as determined by the official procedure (Method 920.159) of the Association of Official Analytical Chemists (AOAC, 1990). FAMEs of the menhaden oil were prepared by transesterification according to American Oil Chemists' Society Method Ce 2-66 (AOCS, 1989).

Preparative HPLC. Two C_{18} reversed-phase columns (Waters, 5.7 × 30 cm), connected in series with a Waters Prep LC 500 system, were used. The test sample was dissolved in acetone. The solvent was acetonitrile at 37 mL/min.

GC/IR Instrumentation. GC separations were performed on a Hewlett-Packard Model 5890 instrument (Avondale, PA) equipped with a flame ionization detector (FID) and a Hewlett-Packard Model 3392A integrator. A 50 m × 0.22 mm (i.d.) CP-Sil-88 capillary column (Chrompack, Inc., Bridgewater, NJ) with a 0.19-μm stationary phase film was used. Helium containing 1.5% argon (Matheson Gas Products, Secaucus, NJ) at approximately 27 cm/s linear velocity was used as the carrier gas, and helium (99.995%) at 30 mL/min was used as the makeup gas to

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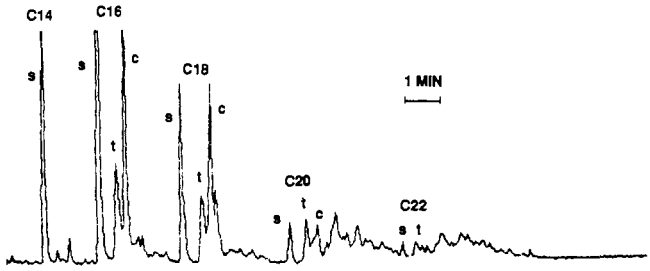

Figure 1. Partial GC/FID chromatogram for a hydrogenated menhaden oil (IV 78) FAME mixture. The "humps" are due to the partial or total overlap of mixture component peaks: s, saturated, t, *trans*; c, *cis*.

the detector. The injector and detector temperatures were 250 and 300 °C, respectively. The carrier gas mixture was purified by using a Hydro-Purge II filter (Alltech Associates) and a heated gas purifier filter (Supelco, Bellefonte, PA). The injection mode was splitless, and a 10-μL Hamilton 701N syringe was used. Injections of about 1 μL were made 6 s after the start of a run, and the total injection time was 15 s. The injector was purged 1 min after the start of the run. The initial column oven temperature was 75 °C with a 2-min hold, followed by a 20 °C/min increase to 240 °C, and the oven was held at this temperature until the analysis was completed.

A Mattson Instruments, Inc. (Madison, WI), Model Sirius 100 FT-IR spectrometer equipped with an MI Cryolect interface operating at 12 K under vacuum was used. This system has been described in detail (Bourne et al., 1984; Reedy et al., 1985). The MI method involved adding argon (1.5% by volume) to the GC carrier gas (helium) and trapping the effluent on the outer rim of a slowly rotating (at about 3 mm/min) gold disk held at cryogenic temperatures. During a run, helium was removed by vacuum pumps, and the analyte molecules surrounded by an excess of argon atoms were frozen into a solid matrix. The analytes isolated in the IR-transparent argon matrix were subsequently analyzed by IR spectroscopy. The position of each analyte peak on the Cryolect collection disk was indexed by its observed GC retention time. Procedures were detailed previously (Mossoba et al., 1989) for reproducibly locating a peak maximum on the collection disk and for optimizing the performance of the system. These latter procedures, which include optical alignment, can minimize the extent of postcolumn peak broadening.

GC/MI/FT-IR Analysis. Three hundred analyte interferograms were coadded (2 min 43 s at 4-cm^{-1} resolution), and the background (300 scans) was usually collected before or after the analyte peak.

RESULTS AND DISCUSSION

FAMEs from hydrogenated menhaden oil [iodine value (IV) 78] exhibited a chromatogram (Figure 1) consisting of numerous overlapping GC peaks on an unresolved background, indicating the presence of a large number of closely eluting or coeluting compounds in this mixture. Retention time and normalized area percent for the *trans*-monounsaturated and saturated FAME GC peaks are given in Table I.

To quantitate the levels of *trans*-monounsaturated and saturated FAMEs in hydrogenated menhaden oil, an HPLC separation was carried out (Figure 2). The components in each of the five HPLC fractions (A–E) collected were subsequently separated by GC, and the identities of the individual *trans*-monounsaturated FAME geometric isomers and saturated FAMEs were confirmed and their levels were quantified by FT-IR.

The GC chromatograms (Figure 3) of the different HPLC fractions indicate that *trans*-monoene FAME GC peaks partially overlap those of adjacent *cis*-monoene FAMEs and that GC peak area may not accurately reflect analyte composition.

Figure 4 shows the IR spectra obtained for these *trans*

Table I. GC Peak Retention Time and Normalized Area Percent for Saturated and *trans*-Monounsaturated FAMEs from Partially Hydrogenated Menhaden Oil (IV 78)

FAME	GC retention time (±0.01) at 240 °C, min	normalized[a] GC peak area, %
14:0	7.95	13.27
16:0	9.42	27.87
t-16:1	9.87	5.62
18:0	11.65	6.61
t-18:1	12.14	3.89
20:0	14.49	1.28
t-20:1	14.92	1.98
22:0	17.51	0.399
t-22:1	17.87	0.665

[a] With respect to all FAMEs from hydrogenated menhaden oil.

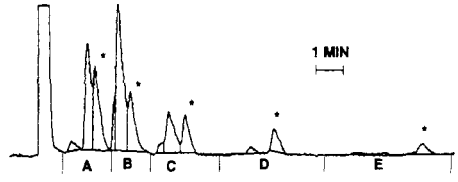

Figure 2. HPLC chromatogram for FAMEs separated on an analytical column. Vertical bars show where cuts were made for collecting fractions A–E; FAEE elution order: *c*-16:1, *c*-18:1, *c*-20:1, *c*-22:1, and *c*-24:1, respectively. Asterisks denote the FAEE internal standard peaks. The preparative-scale HPLC separation time was 6 h.

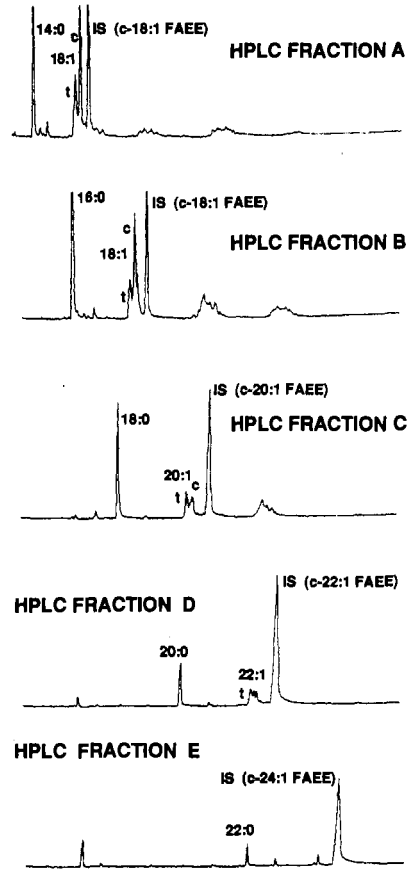

Figure 3. Partial GC/FID chromatograms for FAMEs collected in HPLC fractions A–E.

species. The normalized intensity of the CH_2 asymmetric stretching vibration (2935 cm^{-1}) relative to that of the ester carbonyl band at 1754 cm^{-1} is directly proportional to FAME chain length as follows: 1.2 (*t*-16:1), 1.5 (*t*-18:1), 1.8 (*t*-20:1), and 2.1 (*t*-22:1). Although both *cis* and *trans* FAME geometric isomers exhibit many bands at the same wavenumbers (Mossoba et al., 1990), the weak but sharp

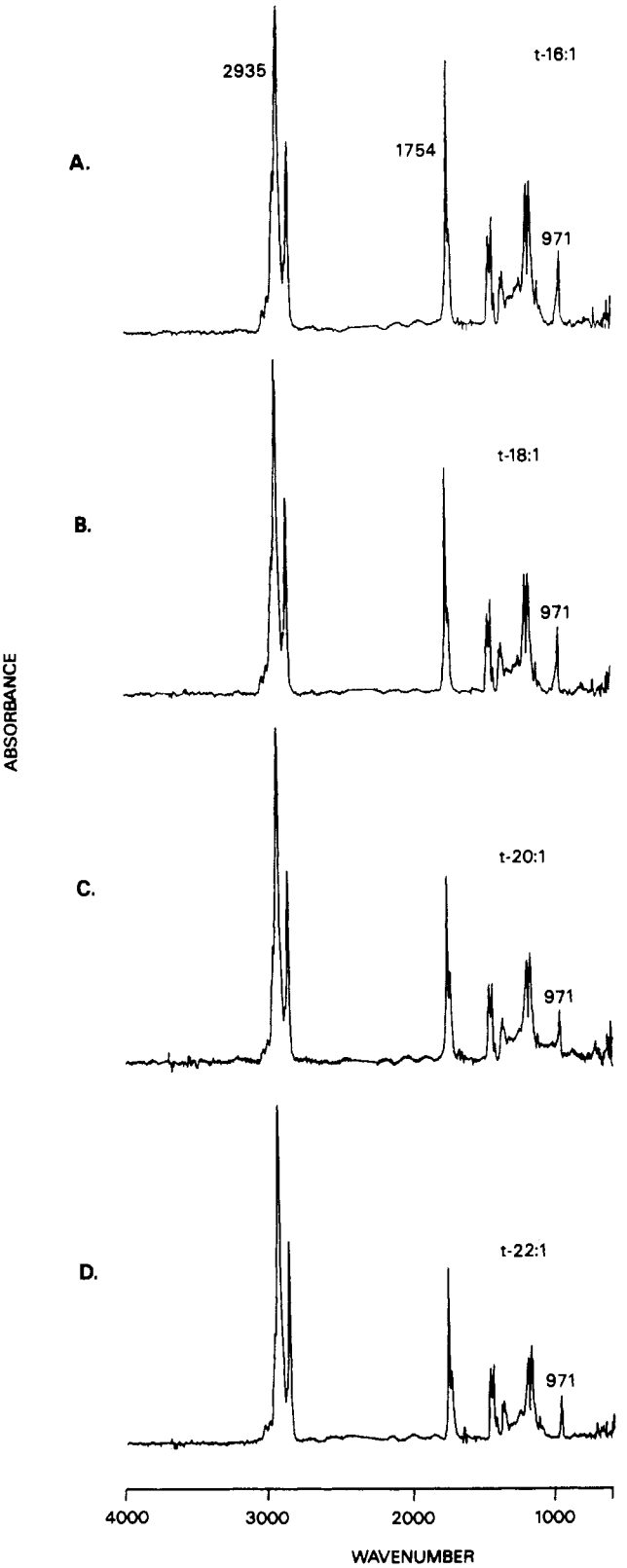

Figure 4. MI/FT-IR spectra at 4-cm^{-1} resolution for (A) *trans*-16:1, (B) *trans*-18:1, (C) *trans*-20:1, and (D) *trans*-22:1 FAMEs. The unique band at 971 cm^{-1} was not observed for the corresponding *cis*-geometric isomers.

band at 971 cm^{-1} is unique to all of the monounsaturated positional isomers with *trans* configuration and was used to quantitate their levels. An advantage of using IR (971 cm^{-1}) instead of GC for quantitation is that any interferences, particularly those due to partial overlap of adjacent *trans* and *cis* isomer GC peaks, would not interfere with the accuracy of the measurements.

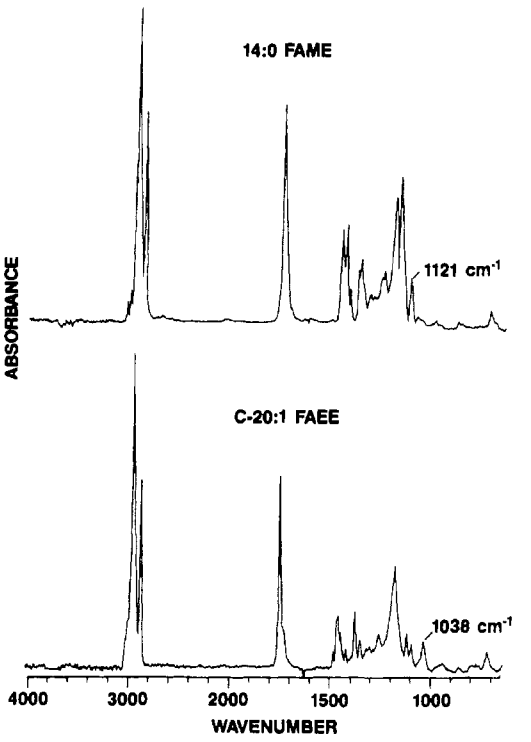

Figure 5. MI/FT-IR spectra at 4-cm^{-1} resolution for (top) 14:0 FAME (monitored band: 1121 cm^{-1}) and (bottom) *cis*-20:1 FAEE internal standard (monitored band: 1038 cm^{-1}).

Table II. Results of Linear Regression Analysis

analyte	slope[a]	*Y*-intercept	r^2
t-16:1 FAME	1.02	2.71	0.998
t-18:1 FAME	0.62	7.86	0.999
t-20:1 FAME	0.31	3.47	0.999
t-22:1 FAME	0.098	−0.077	0.999
14:0 FAME	1.44	9.86	0.997
16:0 FAME	0.91	−13.20	0.999
18:0 FAME	0.38	−7.08	0.999
20:0 FAME	0.13	−1.36	0.972
22:0 FAME	0.041	−0.87	0.976
c-16:1 FAEE	2.20	−1.87	0.994
c-18:1 FAEE	1.25	−13.55	0.983
c-20:1 FAEE	0.25	−2.18	0.982
c-22:1 FAEE	0.10	−0.53	0.998
c-24:1 FAEE	0.091	1.96	0.998

[a] Slope of milliabsorbance vs nanograms of analyte. FT-IR bands monitored were 971, 1121, and 1038 cm^{-1} for *trans*-FAMEs, saturated FAMEs, and *cis*-FAEE internal standards, respectively.

Quantitation was based on measurement of the height of the observed CH out-of-plane deformation band (971 cm^{-1}) for *trans* R_1—HC=CH—R_2 groups and the CH_3 in-plane rocking band (1121 cm^{-1}) for saturated FAMEs (Figure 5). Calibration plots of absorbance (in units of $10^{-3}A$) vs nanograms injected were generated for the 3–102-ng range.

The data for the different calibration sets used were accumulated during a 2-week period when the hydrogenated menhaden oil test samples were analyzed. The regression line parameters for the 14 *trans*-monoene and saturated FAME standards are given in Table II.

Because the *trans* and saturated FAMEs were quantitated in the nanogram range, this method is perhaps independent of the type of GC/IR interface used. The minimum identifiable quantity (MIQ) of analyte is in the subnanogram range with either the direct deposition (DD) interface (Bourne et al., 1990) or the MI interface used in the present study and in the low nanogram range with the light pipe (LP) interface (Cooper and Taylor, 1984; Sebedio et al., 1987).

Table III. Recovery of FAMEs from Partially Hydrogenated Menhaden Oil (IV 78), Based on GC/MI/FT-IR Determination of Fatty Acid Ethyl Ester (FAEE) Internal Standards (IS)

FAEE IS	HPLC fraction	observed mA at 1038 cm^{-1}	GC/MI/FT-IR quantitation, ng	total mg found	true mg	recov, %
c-16:1	A	53.0	24.9	205.0	216.3	94.8
c-18:1	B	16.0	23.7	174.3	207.4	84.1
c-18:1[a]	B	17.3	24.8	182.2	207.4	87.8
c-20:1	C	17.5	79.7	214.1	224.8	95.2
c-20:1[a]	C	15.9	73.2	196.7	224.8	88.0
c-22:1	D	9.5	99.3	124.0	198.9	62.3
c-24:1	E	2.6	6.9	84.9	103.6	82.0

[a] Repeat analysis.

Table IV. GC/MI/FT-IR Quantitation of *trans*-Monounsaturated FAMEs from Partially Hydrogenated Menhaden Oil (IV 78)

FAME	HPLC fraction	observed mA at 971 cm^{-1}	GC/MI/FT-IR quantitation, ng	recov,[a] %	% wt[b]
t-16:1	A	22.1	18.4	94.8	6.1
t-18:1	B	12.5	7.5	84.1	2.5
t-18:1[c]	B	13.0	8.3	87.8	2.7
t-20:1	C	6.5	9.6	95.2	1.05
t-20:1[c]	C	6.4	9.5	88.0	1.12
t-22:1	D	0.60	6.9	62.3	0.54

[a] Values calculated from Table II data. [b] Corrected for recovery. [c] Repeat analysis.

Table V. GC/MI/FT-IR Quantitation of Saturated FAMEs from Partially Hydrogenated Menhaden Oil (IV 78)

FAME	HPLC fraction	observed mA at 1121 cm^{-1}	GC/MI/FT-IR quantitation, ng	recov,[a] %	% wt[b]
14:0	A	62.8	36.7	94.8	12.3
16:0	B	62.4	82.9	84.1	28.0
16:0[c]	B	61.5	82.0	87.8	26.6
18:0	C	17.3	63.5	95.2	6.9
18:0[c]	C	17.3	63.5	88.0	7.5
20:0	D	6.6	60.2	62.3	1.17
22:0	E	1.8	64.9	82.0	0.37

[a] Values calculated from Table II data. [b] Corrected for recovery. [c] Repeat analysis.

Recovery was based on the determination of *cis*-monunsaturated fatty acid ethyl ester (FAEE) internal standards (IS). Calibration plots of milliabsorbance (monitored at 1038 cm^{-1}) vs nanograms injected were generated for FAEE IS. The 1038-cm^{-1} band (Figure 5) is not present in MI/FT-IR spectra of FAMEs.

The amount of analyte present in injected aliquots was calculated from the observed absorbance values (Tables III–V), and the corresponding linear regression equation describing the calibration plot. Recovery (Table III) and weight percent of *trans*-monoene (Table IV) and saturated (Table V) FAMEs were calculated as

$$\text{recovery } (\%) = w_i(V_p/v_i)(W_{LC}/W_p)(100/W_{IS})$$

$$\text{weight } \% = w_i(V_p/v_i)(W_{LC}/W_p)(100/W_{Mix})$$

where w_i is the weight of analyte (FAME or IS) present in injected aliquot; v_i is the volume of injected aliquot taken from test solution; V_p is the volume of test solution; W_p is the weight of test portion of pure analyte obtained from the HPLC fraction; W_{LC} is the weight of pure analyte obtained from the HPLC fraction; W_{IS} is the weight of IS used; and W_{Mix} is the weight of FAME mixture obtained by partially hydrogenating and esterifying refined menhaden oil.

Table VI. Weight Percent of Saturated FAMEs from Partially Hydrogenated Menhaden Oil

FAME	present study[a]	lit. data[b]	lit. data[c]
14:0	12.3	10.5	10.5[d]
16:0	28.0	24.1	26.6
16:0[e]	26.6		
18:0	6.9	5.2	9.7
18:0[e]	7.5		
20:0	1.17	0.7	2.9
22:0	0.37	0.3	1.0

[a] Values obtained from Table V (IV 78). [b] Sebedio and Ackman (1983) (IV 84.5). [c] Beare-Rogers (1979) (IV not reported). [d] Value includes contribution from 14:1. [e] Repeat analysis.

Satisfactory repeatability was achieved in the duplicate quantitation of HPLC fractions B and C (Tables IV and V); *trans*-18:1 and *trans*-20:1 FAME weight percent values varied by 5.6 and 6.3%, respectively. Those for 16:0 and 18:0 FAMEs varied by 7.5 and 7.7%, respectively. Differences between GC peak area determination (Table I) and this GC/MI/FT-IR quantitation method (Tables IV and V) are attributed to the lack of specificity of the GC approach, which assumes that each GC peak is always baseline-resolved and due to a single analyte.

There is a paucity of publications on quantitation of saturated and monounsaturated fatty acids in partially hydrogenated menhaden oil. Two reports were found, one by Sebedio and Ackman (1983) and one by Beare-Rogers (1979); only the former provided data on *t*-20:1 (i.e., 2.84% at IV 84.5). The composition found for saturated species from partially hydrogenated menhaden oil is listed with literature values in Table VI. No general comparison could be made of the levels of FAMEs found in partially hydrogenated menhaden oil in the present work and those reported in the literature because of differences in iodine values, oil composition, and hydrogenation conditions.

CONCLUSIONS

GC/MI/FT-IR was used to identify and quantify the *t*-18:1 geometric isomer and related *trans*-monounsaturated and saturated FAMEs in partially hydrogenated menhaden oil.

For accurately quantifying FAMEs, this infrared method is preferred over GC peak area determination because it is based on discriminatory features, namely, absorbance at 971, 1121, and 1040 cm^{-1}, for *trans*-monounsaturated FAMEs, saturated FAMEs, and FAEE internal standards, respectively. In particular, the unique band at 971 cm^{-1} that is observed only for *trans* species inherently excludes any interference from *cis* isomers.

The use of FAEE internal standards was required. At an iodine value of 78, the weight percent of *t*-18:1 in partially hydrogenated menhaden oil was 2.6%. For duplicate determinations of FAMEs, variability in composition was in the range 5–8%.

ACKNOWLEDGMENT

We thank Curtis Barton for statistical analysis and Hubert S. Lin and Sebastian Cianci for technical assistance.

LITERATURE CITED

AOAC. *Official Methods of Analysis*, 15th ed.; Association of Official Analytical Chemists: Arlington, VA, 1990; Method 920.159.

AOCS. *American Oil Chemists' Society Official Methods*; American Oil Chemists' Society: Champaign, IL, 1989; Method Ce 2–66.

AOCS. American Oil Chemists' Society Netherlands Study Puts *Trans* in Spotlight Again. *INFORM* **1990**, *1* (10), 875–878.

Beare-Rogers, J. L. Partially Hydrogenated Rapeseed and Marine Oils. *Geom. Positional Fatty Acid Isomers* **1979**, *6*, 131–149.

Bourne, S.; Reedy, G.; Coffey, P.; Mattson, D. Matrix Isolation GC/FT-IR. *Am. Lab. (Fairfield, Conn.)* **1984**, *16*, 90–101.

Bourne, S.; Hoefner, A. M.; Norton, K. L.; Griffiths, P. R. Performance Characteristics of a Real-Time Direct Deposition Gas Chromatography/Fourier Transform Infrared Spectrometry System. *Anal. Chem.* **1990**, *62*, 2448–2452.

Cooper, J. R.; Taylor, L. T. Establishing Identification Limits of Model Compounds in Capillary Gas Chromatography/Fourier Transform Infrared Spectrometry. *Anal. Chem.* **1984**, *56*, 1989–1993.

Grundy, S. M. Trans Monounsaturated Fatty Acids and Serum Cholesterol Levels. *N. Engl. J. Med.* **1990**, *323* (7), 480–481.

Mensink, R. P.; Katan, M. B. Effect of Dietary *Trans* Fatty Acids on High-Density and Low-Density Lipoprotein Cholesterol Levels in Healthy Subjects. *N. Engl. J. Med.* **1990**, *323* (7), 439–445.

Mossoba, M. M.; Niemann, R. A.; Chen, J.-Y. T. Picogram Level Quantitation of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin in Fish Extracts by Capillary Gas Chromatography/Matrix Isolation/Fourier Transform Infrared Spectrometry. *Anal. Chem.* **1989**, *61* (15), 1678–1685.

Mossoba, M. M.; McDonald, R. E.; Chen, J.-Y. T.; Armstrong, D. J.; Page, S. W. Identification and Quantitation of *trans*-9, *trans*-12 Octadecadienoic Acid Methyl Ester and Related Compounds in Hydrogenated Soybean Oil and Margarines by Capillary Gas Chromatography/Matrix Isolation/Fourier Transform Infrared Spectroscopy. *J. Agric. Food Chem.* **1990**, *38*, 86–92.

Ratnayake, N. W. M.; Beare-Rogers, J. L. Problems of Analyzing C_{18} *Cis* and *Trans*-Fatty Acids of Margarine on the SP-2340 Capillary Column. *J. Chromatogr. Sci.* **1990**, *28*, 633–639.

Reedy, G. T.; Ettinger, D. C.; Schneider, J. F.; Bourne, S. High Resolution Gas Chromatography/Matrix Isolation Infrared Spectrometry. *Anal. Chem.* **1985**, *57*, 1602–1609.

Sebedio, J. L.; Ackman, R. G. Hydrogenation of Menhaden Oil. *J. Am. Oil Chem. Soc.* **1983**, *60* (12), 1986–1991.

Sebedio, J. L.; LeQuere, J. L.; Semon, E.; Morin, O.; Prevost, J.; Grandgirand, A. A Heat Treatment of Vegetable Oils. II. GC-MS and GC-FTIR Spectra of Some Isolated Cyclic Fatty Acid Monomers. *J. Am. Oil Chem. Soc.* **1987**, *64*, 1324–1333.

Willett, W. C.; Stampfer, M. J.; Manson, J. E.; Colditz, G. A.; Speizer, F. E.; Rosner, B. A.; Sampson, L. A.; Hennekens, C. H. Intake of *Trans* Fatty Acids and Risk of Coronary Heart Disease Among Women. *Lancet* **1993**, *341*, 581–585.

Received for review May 25, 1993. Revised manuscript received August 20, 1993. Accepted September 7, 1993.[⊗]

[⊗] Abstract published in *Advance ACS Abstracts*, October 15, 1993.