# Spectral Confirmation of *Trans* Monounsaturated $C_{18}$ Fatty Acid Positional Isomers[1]

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**ABSTRACT:** The *trans* octadecenoic acid methyl ester isomers were obtained from a partially hydrogenated soybean oil sample and isolated by silver-ion high-performance liquid chromatography. The double bond configuration was confirmed to be *trans* by using gas chromatography–direct deposition–Fourier transform infrared spectroscopy. The double bond positions for nine individual *trans* octadecenoic acid positional isomers were confirmed by gas chromatography–electron ionization mass spectrometry after derivatization to 2-alkenyl-4,4-dimethyloxazoline. These nine *trans* positional isomers were resolved on either one of the two polar 100% cyanopropylpolysiloxane 100-m capillary columns, SP 2560 and Cp-Sil 88, at an isothermal temperature of 140°C. These nine isomers were confirmed to have double bonds at carbons C-8 through C-16. The pair of *trans* octadecenoic acid positional isomers with double bonds at C-13 and C-14 are reported for the first time to be resolved by gas chromatography.
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**KEY WORDS:** 4,4-Dimethyl oxazoline (DMOX), direct deposition, *trans* double bonds, fatty acid methyl esters (FAME), gas chromatography, infrared spectroscopy, mass spectrometry, monounsaturated fatty acids, positional isomers.

*Trans* fatty acids occur naturally in animal fats as a result of biohydrogenation; they are also found in many food products that contain partially hydrogenated vegetable oils (1). The nutritional significance of *trans* fatty acids has led to increased interest in accurate and rapid methods for quantitating the total *trans* fatty acid content of foods (2). There is also interest in procedures (3) that can separate and confirm the double bond configuration and position for individual *trans* fatty acid positional isomers.

Capillary gas chromatography (GC) has been the standard tool for the separation of fatty acids (usually as fatty acid methyl esters, FAME) (4). However, the peaks of several *trans* and *cis* octadecenoic acid (18:1) positional isomers overlap (Fig. 1A), even with GC columns that have polar stationary phases (5). Nevertheless, the complete separation of 18:1 *trans* from *cis* geometric isomers was achieved by silver-ion thin-layer chromatography (TLC) (6–9) or by silver-ion high-performance liquid chromatography (HPLC) (3,10). The subsequent separation of 18:1 positional isomers could then be carried out by GC (3,7–10). Some 18:1 positional isomers were also separated by $Ag^+$–HPLC (3,10).

In 1995, four reports in the literature (7–10) detailed the separation of 18:1 fatty acid isomers by $Ag^+$–TLC (7–9) or $Ag^+$–HPLC (10), followed by capillary GC on polar phases. For some of the observed *trans* 18:1 positional isomer GC peaks, the same peak was assigned to a different *trans* positional isomer in three of these four publications (7–10). Therefore, the need to confirm the identity of individual *trans* 18:1 positional isomers unequivocally is timely.

In the present study, the double bond configuration and position were confirmed for individual *trans* 18:1 fatty acid positional isomers by using spectral techniques. Several fractions, including the one containing the *trans* monounsaturated $C_{18}$ FAME, were isolated by $Ag^+$–HPLC. The double bond configuration for the FAME in the *trans* 18:1 HPLC fraction was confirmed by using GC–direct deposition–Fourier transform infrared (DD–FTIR) spectroscopy (11). A portion of this HPLC fraction was converted to the 2-alkenyl-4,4-dimethyloxazoline (DMOX) derivative (12,13) and used to confirm the double bond position for individual *trans* 18:1 FAME positional isomers by GC–electron ionization mass spectrometry (EIMS).

## MATERIALS AND METHODS

Hydrogenated soybean oil was donated by Dr. Wayne E. Emmons of SGS Control Services, Inc. (Deer Park, TX). All reagents and solvents were reagent-grade and supplied by Nu Check Prep, Inc. (Elysian, MN) and Fisher (Pittsburgh, PA). Previously described detailed procedures (2) were followed for the preparation of FAME. The Δ6, Δ7, Δ9, Δ11, Δ12, Δ13,

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and Δ15 *trans* 18:1 FAME positional isomer standards were purchased from Sigma Chemical Co. (St. Louis, MO).

The method of Fay and Richly (13) for derivatizing FAME to DMOX was modified as follows. About 150 µL 2-amino-2-methyl-1-propanol was added to 24 mg neat methyl esters in a 4-mL reaction vial. The vial was suspended in a wax bath held at 170°C for 6 h. The contents of the vial were cooled and transferred with 5 mL methylene chloride to a 250-mL separatory funnel that contained 40 mL petroleum ether. The funnel contents were shaken, and the petroleum ether layer was washed with 40 mL deionized water and then dried with sodium sulfate. The solution was evaporated under a stream of argon, and the residue was dissolved in isooctane.

HPLC separations were performed with a Waters 600E solvent delivery system (Waters Associates, Milford, MA), a Rheodyne 7125 injector (Rheodyne, Inc., Cotati, CA) with a 20-µL injection loop, and a Waters Model 996 photodiode array detector (Waters Associates). A ChromSpher Lipids column (4.6 mm i.d. × 250 mm stainless steel; 5 µ particle size; silver impregnated) was acquired from Chrompack, Inc. (Bridgewater, NJ). Solvent flow was set at 1.0 mL/min. The mobile phase was 0.3% acetonitrile in hexane (isocratic). The test portion size was 100 µg FAME.

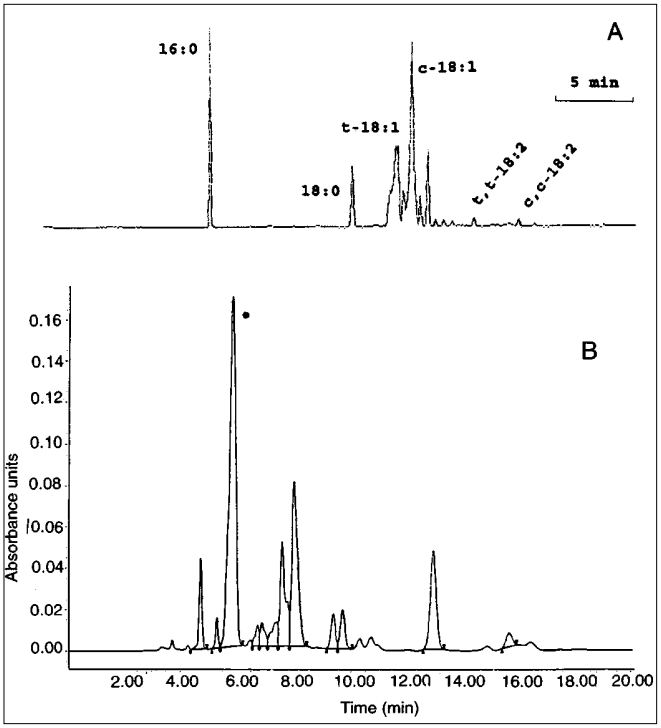


**FIG. 1.** (A) Capillary gas chromatogram for a complex mixture of partially hydrogenated soybean oil fatty acid methyl esters (FAME) in which several *trans* and *cis* 18:1 positional isomers peaks overlap. (B) Separation of same mixture of FAME by silver ion–high-performance liquid chromatography (HPLC). Column: ChromSpher Lipids. Solvent flow: 1.0 mL/min 0.3% acetonitrile in hexane (isocratic). Test portion size: 100 µg FAME. Detection: ultraviolet (UV) 206 nm. The *trans* 18:1 FAME isomers in this partially hydrogenated soybean oil test sample gave rise to the most intense HPLC peak (denoted with an asterisk).

Semipreparative HPLC fractionations were made under similar conditions with a 10-mm i.d. × 250 mm ChromSpher Lipids column and a flow rate of 5 mL/min.

For the GC–DD–FTIR analysis, the GC separation was performed on a Hewlett-Packard Model 5890 Series II instrument (Avondale, PA). A 50 m × 0.22 mm (i.d.) CP-Sil 88 capillary column (Chrompack, Inc.) with a 0.19-µm stationary phase film was used. The GC experimental conditions were: splitless injection; initial temperature, 70°C; initial time, 2 min; purge delay time, 1 min; ramp at 20°C per min; final temperature, 200°C. An FTS-60A FT-IR spectrometer (Bio-Rad, Digilab Division, Cambridge, MA), equipped with a direct deposition Tracer™ interface operating near 77K under vacuum, was used. This system has been described in detail (11).

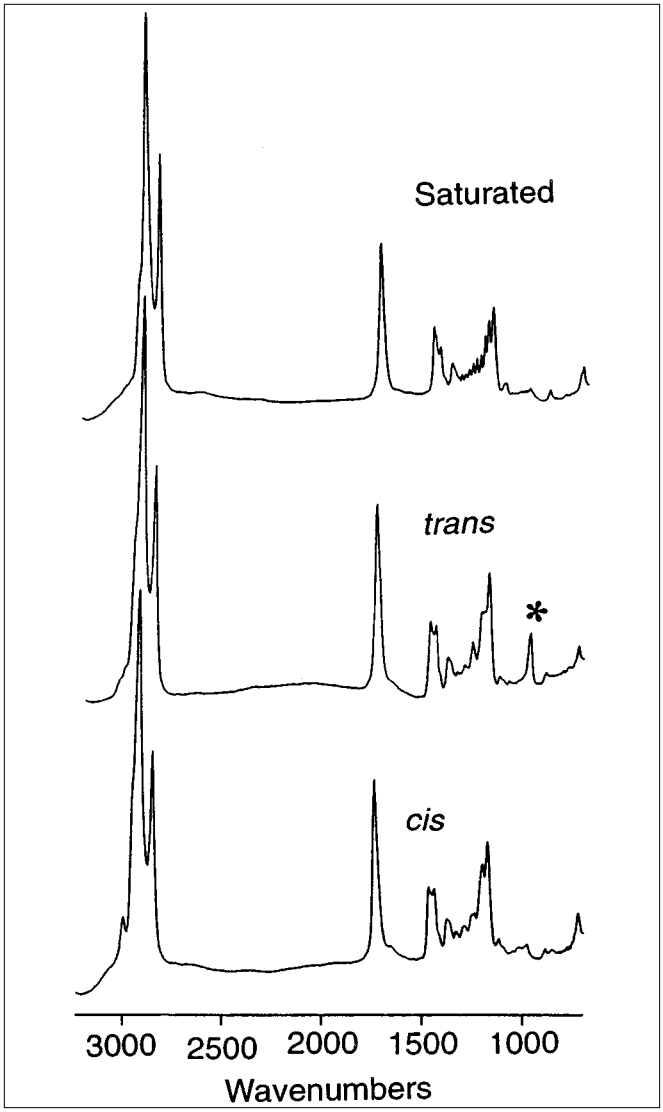


**FIG. 2.** Gas chromatography–direct deposition–Fourier transform infrared spectrum recorded at nearly liquid nitrogen temperature under vacuum for the *trans* 18:1 FAME isomers exhibited a C–H out-of-plane deformation band at 966 $cm^{-1}$ (denoted by an asterisk). This unique band is not found in the spectra of saturated and *cis* unsaturated FAME standards shown for comparison. See Figure 1 for abbreviation.

Low-resolution GC-EIMS analyses were performed with a Hewlett-Packard 5890 series II gas chromatograph, coupled to a Fisons VG (Wythenshawe, United Kingdom) Autospec Q mass spectrometer and OPUS 4000 data system. The GC/MS system was used with version 2.1BX software. The capillary GC columns used were the CP-Sil 88 (Chrompack, Inc.), 100 m × 0.22 mm (i.d.), with a 0.19-µm stationary phase film, and the SP2560 (Supelco, Bellefonte, PA), 100 m × 0.25 mm (i.d.) with a 0.20-µm film thickness. The GC/MS conditions were as follows: hydrogen carrier gas; split injection; head pressure, 20 psi; oven temperature, 140°C (isothermal); split vent flow rate, 30 mL/min; septum purge flow rate, 40 mL/min; linear velocity, 32 cm/s; split ratio, 23:1; and injector and transfer lines held at 220 and 200°C, respectively. The same chromatographic resolution was obtained with both columns. The mass spectrometer was tuned to a resolution of 1400 (5% valley) by observing *m/z* 281 in the EI mass spectrum of perfluorokerosene (PFK). The mass scale was calibrated with PFK for magnet scans from 360 to 60 daltons at 0.5 s/decade. The filament emission was 200 µA at 70 eV. The ion source temperature was 250°C.

## RESULTS AND DISCUSSION

The observed $Ag^+$–HPLC chromatogram for FAME from a partially hydrogenated soybean oil test portion is shown in Figure 1B. The total *trans* 18:1 FAME level for this oil was previously determined by vibrational spectroscopy (2) to be relatively high (about 38%). The most intense HPLC peak (Fig. 1) was confirmed to be due to the *trans* 18:1 geometric isomer by using GC–DD–FTIR spectroscopy. The unique band at 966 $cm^{-1}$, characteristic of double bonds with *trans* geometry (2), is absent from the spectra found for either saturated or *cis* unsaturated FAME shown in Figure 2 for comparison.

The fatty acid DMOX derivative was used for the GC–EIMS analyses for two major reasons: (i) the resulting chromatographic resolution is usually as good as, and at times, superior to that obtained for FAME on the same 100% cyanopropyl polysiloxane polar capillary GC stationary phase (14); and (ii) distinctive mass spectra are usually obtained, thus making their interpretation relatively simple (12–14).

Under our reaction conditions, the conversion from FAME to DMOX derivatives was not quantitative. The presence of both derivatives in the same mixture allowed monitoring and comparison of their GC characteristics (in particular, resolution) under identical GC conditions. The *trans* 18:1 FAME eluted about 1 h before the DMOX derivatives at 140°C (isothermal) (see Fig. 3). The data presented in Figure 3 are for two separate GC runs that differed only in the injection volume, 0.4 and 1.0 µL, shown in the top and bottom traces, respectively. For each of these two GC runs, the *m/z* 264 and

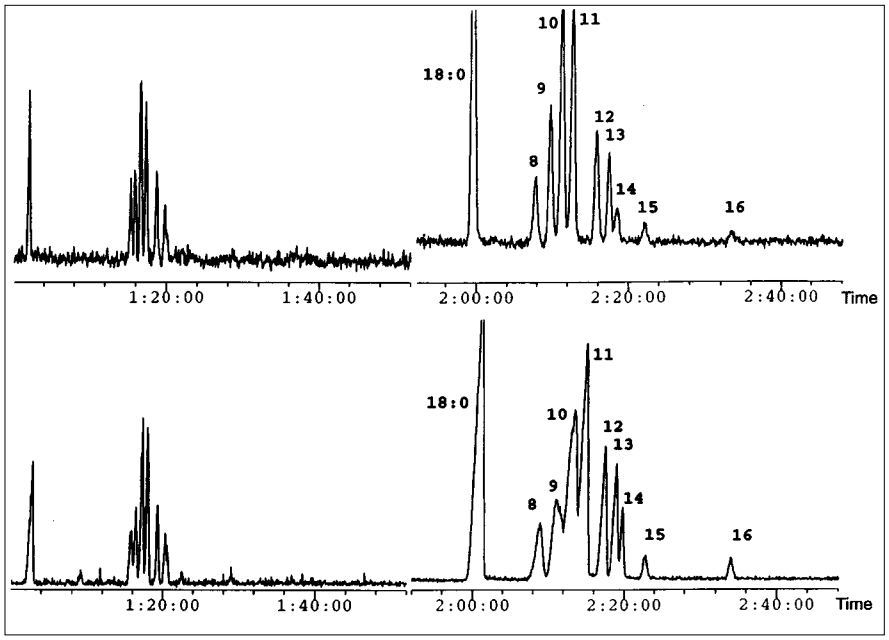


**FIG. 3.** Gas chromatography–electron ionization mass spectrometry (GC–EIMS) chromatographic data for two separate GC runs with the SP 2560, 100-m capillary column. A similar resolution was obtained with the Cp-Sil 88, 100-m capillary column under identical experimental conditions. The top and bottom traces were for two GC runs in which 0.4 and 1.0 µL portions of the test sample were injected, respectively. The *m/z* 264 and 113 ion profiles were recorded for *trans* 18:1 positional isomer FAME and 4,4-dimethyloxazoline (DMOX) derivatives, respectively, present in the same test portion. The DMOX eluted about 1 h after the FAME derivatives under 140°C isothermal GC conditions. The numerical labels 8 through 16 next to GC peaks denote the Δ8 through Δ16 *trans* 18:1 positional isomers. See Figure 1 for abbreivation.

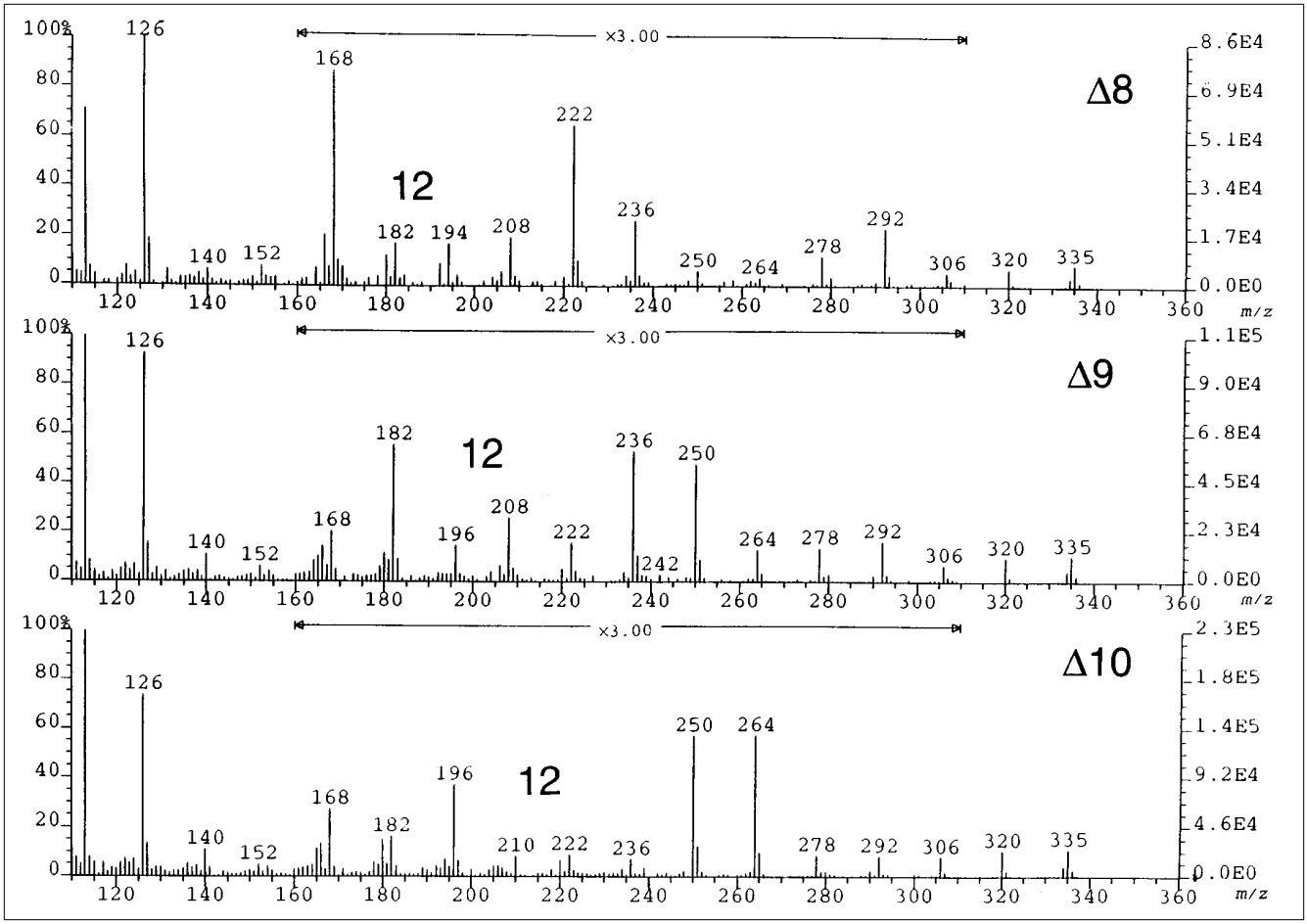


**FIG. 4.** GC–EIMS spectra observed for the Δ8, Δ9, and Δ10 *trans* 18:1 DMOX positional isomers; a gap of 12 mass units was observed between the pairs of adjacent ions at *m/z* 182 and 194; 196 and 208; and 210 and 222, respectively; the ions due to allylic cleavage were found at *m/z* 168, 222, and 236; 182, 236, and 250; and 196, 250, and 264, respectively. See Figure 3 for abbreviations.

113 ion profiles, recorded for the *trans* 18:1 FAME and DMOX positional isomers, appeared in this same sequence, respectively. Inspection of the bottom GC profile (1.0-μL injection) shows evidence of overload in the early part of the *m/z* 113 DMOX trace, whereas the response of the Δ13 and Δ14 pair of positional isomers (in the second half of this trace) appears to be enhanced. This is the first capillary GC report of the separation of these two *trans* 18:1 positional isomers.

A fatty acid DMOX mass spectrum consists of a series of *even*-mass ions, separated by a mass difference of 14 due to the loss of a methylene group (12). A gap of 12 mass units between ions containing $n - 1$ and $n$ carbon atoms indicates the presence of a double bond between carbons $n$ and n + 1 of the parent fatty acid. More abundant peaks due to allylic cleavage, corresponding to ions containing $n - 2$, $n + 2$, and $n + 3$ carbons of the parent fatty acid, flank the pair of diagnostic ions that are separated by a gap of 12 mass units (12). Deviations from these rules are found in DMOX spectra when a double bond is closer to the extremities of the fatty acid molecule (e.g., Δ6 and Δ7, *vide infra*). The mass spectral data (Figs. 4 and 5) confirmed the double bond position along the hydrocarbon chain for nine individual *trans* 18:1 positional isomers, each having a double bond at a carbon located between C-8 and C-16. The identities of the Δ9, Δ11, Δ12, Δ13, and Δ15 *trans* 18:1 positional isomers were further confirmed by comparison with standards.

For partially hydrogenated fats, 18:1 positional isomers are usually produced as a distribution in which the abundance of an isomer generally decreases as its double bond gets closer to either end of the fatty acid chain (6). Accordingly, the levels of the Δ4–Δ7 *trans* 18:1 isomers are expected to be low and difficult to identify. The Δ6 and Δ7 *trans* 18:1 FAME positional isomer standards were commercially acquired and analyzed by GC under identical experimental conditions. As FAME, the Δ6 and Δ7 *trans* 18:1 positional isomers coeluted, but they were well resolved as DMOX derivatives (Fig. 6). The mass spectra of Δ6 and Δ7 *trans* 18:1 DMOX positional isomer were unique and permitted identification of these isomers, even though their DMOX mass spectra did not exhibit the expected diagnostic ions. When co-injected with

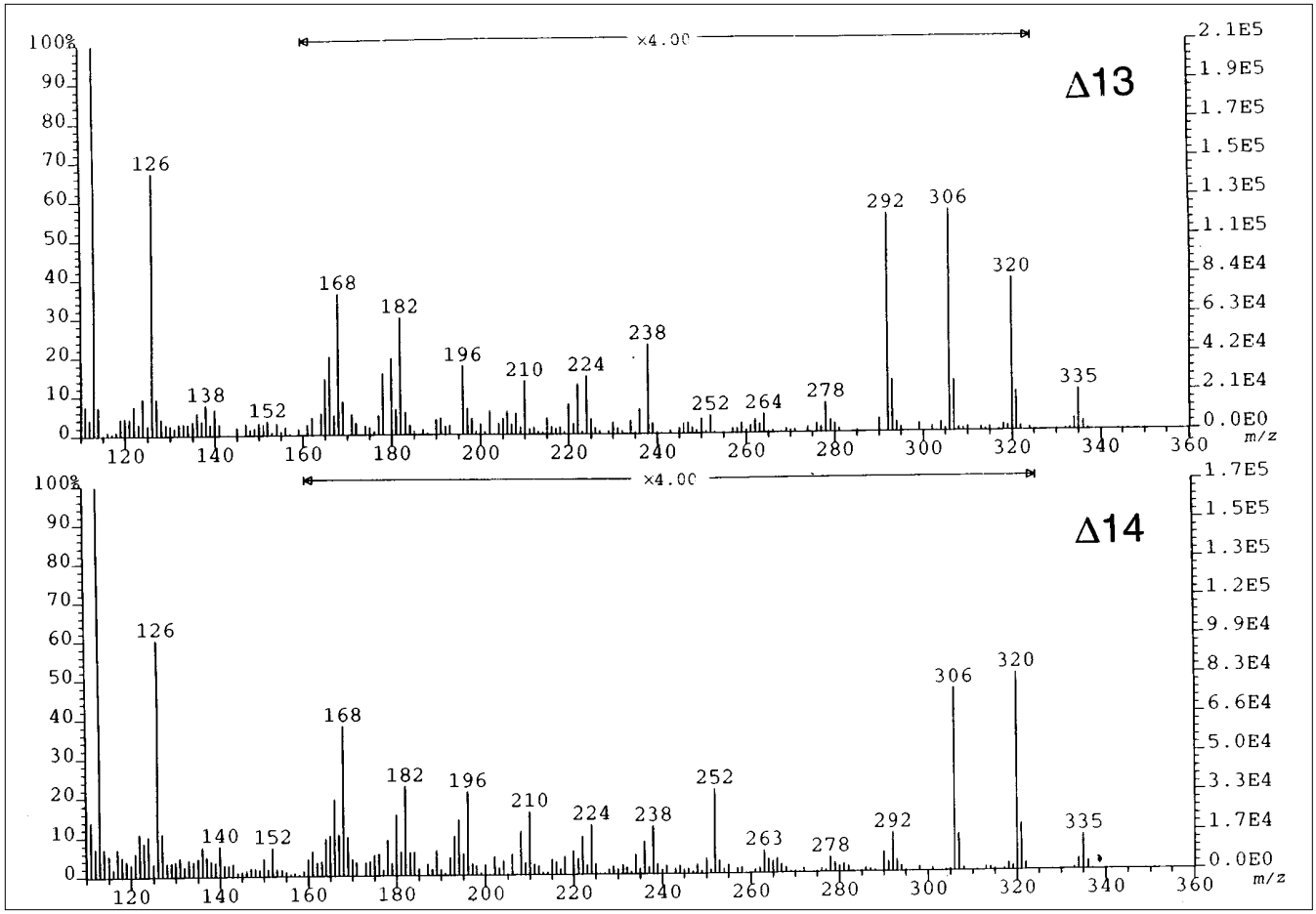


**FIG. 5.** GC–EIMS spectra for the Δ13 and Δ14 pair of *trans* 18:1 DMOX positional isomers; the ions due to allylic cleavage were found at *m/z* 238, 292, and 306; and 252, 306, and 320, respectively. See Figure 3 for abbreviations.

the partially hydrogenated soybean oil test portion analyzed, the Δ6 and Δ7 DMOX isomers co-eluted with the Δ8 and Δ9 DMOX isomers, respectively. Therefore, it was not possible to confirm by GC–EIMS the identity of the minor Δ6 (or Δ7) isomer, expected to be found at a much lower level than that of the Δ8 (or Δ9, respectively) isomer with which it would be co-eluting. By using a trifluoropropylmethyl stationary phase, the Δ6 and Δ7 *trans* 18:1 FAME positional isomers were reportedly (15) resolved by GC after epoxidizing double bonds with peracetic acid.

GC–DD–FTIR and GC–EIMS are complementary techniques that can confirm the double bond configuration and position for individual 18:1 fatty acid isomers.

Better GC resolution was obtained for the DMOX than for the FAME derivatives for the two pairs of Δ6 and Δ7, and Δ13 and Δ14 *trans* 18:1 fatty acid positional isomers.

Unique DMOX mass spectra were observed for 11 individual *trans* 18:1 fatty acid positional isomers with double bonds at carbons C-6 through C-16. The identities were confirmed for the Δ8 through Δ16 individual *trans* 18:1 positional isomers from partially hydrogenated soybean oil.

## REFERENCES

1. Firestone, D., and A. Sheppard, Determination of *trans* Fatty Acids, in *Advances in Lipid Methodology-One,* W.W. Christie (ed.), The Oily Press, Ayr, 1992, pp. 273–322.
2. Mossoba, M.M., M.P. Yurawecz, and R.E. McDonald, Rapid Determination of the Total *trans* Content of Neat Hydrogenated Oils by Attenuated Total Reflection Spectroscopy, *J. Am. Oil Chem. Soc. 73*:1003–1009 (1996).
3. Christie, W.W., and G.McG. Breckenridge, Separation of *cis* and *trans* Isomers of Unsaturated Fatty Acids by High Performance Liquid Chromatography in the Silver Ion Mode, *J. Chromatogr. 469*:261–269 (1989).
4. Christie, W.W., *Gas Chromatography and Lipids: A Practical Guide*, The Oily Press, Ayr, 1990.
5. Duchateau, G.S.M.J.E., H.J. van Oosten, and M.A. Vascocellos, Analysis of *cis*- and *trans*-Fatty Acid Isomers in Hydrogenated and Refined Vegetable Oils by Capillary Gas-Liquid Chromatography, *J. Am. Oil Chem. Soc. 73*:275–282 (1996).
6. Marchand, C.M., Positional Isomers of *trans*-Octadecenoic Acids in Margarines, *Can. Inst. Food Sci. Technol. J. 15*:196–199 (1982).
7. Molkentin, J., and D. Precht, Optimized Analysis of *trans*-Octadecenoic Acids in Edible Fats, *Chromatographia 41*:267–272 (1995).

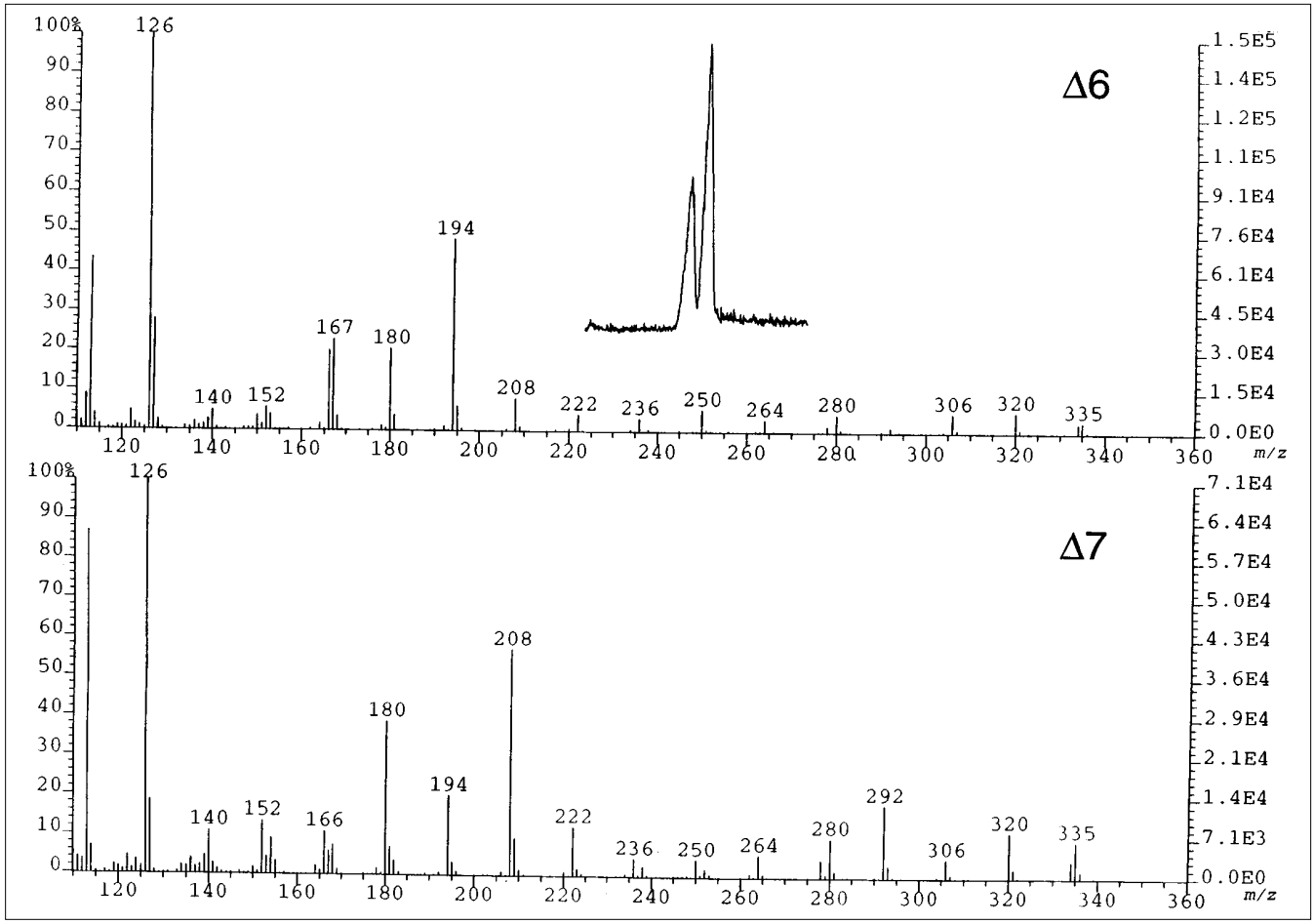


**FIG. 6.** GC-EIMS spectra for Δ6 and Δ7 *trans* 18:1 DMOX positional isomer standards. The inset shows the *m/z* 113 chromatographic trace observed for a co-injected qualitative test mixture of these two standards. See Figure 3 for abbreviations.


8. Precht, D., Variation of *trans* Fatty Acids in Milk Fats, *Z. Ernahrungswiss. 34*:27–29 (1995).
9. Wolff, R.L., and C.C. Bayard, Improvement in the Resolution of Individual *trans*-18:1 Isomers by Capillary Gas–Liquid Chromatography: Use of a 100-m CP-Sil 88 Column, *J. Am. Oil Chem. Soc. 72*:1197–1201 (1995).
10. Adlof, R.O., L.C. Copes, and E.A. Emken, Analysis of the Monoenoic Fatty Acid Distribution in Hydrogenated Vegetable Oils by Silver Ion HPLC, *J. Chromatogr. 72*:571–574 (1995).
11. Bourne, S., A.M. Haefner, K.L. Norton, and P.R. Griffiths, Performance Characteristics of a Real-Time Direct Deposition Gas Chromatography/Fourier Transform Infrared Spectrometry System, *Anal. Chem. 62*:2448–2452 (1990).
12. Zhang, J.Y., Q.T. Yu, B.N. Liu, and Z.H. Huang, Chemical Modification in Mass Spectrometry IV. 2-Alkenyl-4,4-Dimethyl Oxazolines as Derivatives for Double Bond Location of Long-Chain Olefinic Acids, *Biomed. Environ. Mass Spectrom. 15*:33–44 (1988).
13. Fay, L., and U. Richly, Location of Double Bonds in Polyunsaturated Fatty Acids by Gas Chromatography-Mass Spectrometry after 4,4-Dimethyloxazoline Derivatization, *J. Chromatogr. 541*:89–98 (1991).
14. Mossoba, M.M., M.P. Yurawecz, J.A.G. Roach, H.S. Lin, R.E. McDonald, B.D. Flickenger, and E.G. Perkins, Elucidation of Cyclic Fatty Acid Monomer Structures. Cyclic and Bicyclic Ring Sizes and Double Bond Position and Configuration, *J. Am. Oil Chem. Soc. 72*:721–727 (1995).
15. Pfalzgraf, A., and H. Steinhart. Origin of *trans* Fatty Acids and Their Analysis in Food, Tissue, and Blood Plasma, *Fett/Lipid 98(2S)*:81–82 (1996).


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