Evaluation of the CP-Sil 88 and SP-2560 GC Columns Used in the Recently Approved AOCS Official Method Ce 1h-05: Determination of *cis*-, *trans*-, Saturated, Monounsaturated, and Polyunsaturated Fatty Acids in Vegetable or Non-ruminant Animal Oils and Fats by Capillary GLC Method

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ABSTRACT: The AOCS Official Method Ce 1h-05 was recently approved at the 96th AOCS Annual Meeting (2005) by the Uniform Methods Committee as the official method for determining *cis* and *trans* FA in vegetable or non-ruminant fats and oils. A series of experiments was undertaken using a margarine (hydrogenated soybean oil) sample containing approximately 34% total *trans* FA (28% 18:1 *trans*, 6% 18:2 *trans*, and 0.2% 18:3 *trans*), a low-*trans* oil (*ca*. 7% total *trans* FA), and a proposed system suitability mixture (12:0, 9*c*-18:1, 11*c*-18;1, 9*c*,12*c*,15*c*-18:3, 11*c*-20:1, and 21:0) in an effort to evaluate and optimize the separation on the 100-m SP-2560 and CP-Sil 88 flexible fused-silica capillary GC columns recommended for the analysis. Different carrier gases and flow rates were used during the evaluation, which eventually lead to the final conditions to be used for AOCS Official Method Ce 1h-05.

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KEY WORDS: CP-Sil 88, gas chromatography, partially hydrogenated fats, SP-2560, *trans* fatty acid analysis, vegetable oil.

Effective January 1, 2006, the U.S. Food and Drug Administration (FDA) is requiring mandatory nutritional labeling of *trans* FA such that they be declared on the nutrition label of conventional foods and dietary supplements on a separate line immediately under the line for the declaration of saturated FA (1). A similar mandatory labeling requirement went into effect in Canada on December 12, 2005 (2). In an effort to harmonize the methodology used for quantifying *trans* FA, a meeting of invited experts from across the edible oil and food industries, contract laboratories, and U.S. FDA and Health Canada was held in December 2003 at the American Oil Chemists' Society (AOCS) headquarters in Champaign, Illinois. The primary objective of this meeting was to reach a consensus as to the methodology that would be evaluated through collaborative studies for both the chromatography and extraction techniques. The starting point for the development of the method was based on the study done by Ratnayake *et al*. (3). That study examined different GC column oven temperature profiles required to give the best resolution of 13*t*- + 14*t*-18:1 from 9*c*-18:1; near-baseline separation of 16*t*-18:1, 13*c*-18:1, and 14*c*-18:1; plus separation of 11*c*-20:1 from 9*c*,12*c*,15*c*-18:3 (α-linolenic) and its mono-*trans* geometric isomers, especially the 9*t*,12*c*,15*c*-18:3 isomer on both SP-2560 and CP-Sil 88 columns (100 m × 0.25 mm × 0.20 μm). The focus of this work was on optimization of the chromatography developed for the Official Method Ce 1h-05, "Determination of *cis*-, *trans*-, Saturated, Monounsaturated and Polyunsaturated Fatty Acids in Vegetable or Non-ruminant Animal Oils and Fats by Capillary GLC" (4).

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MATERIALS AND METHODS

FA analyses by GC coupled to FID. A FAME mixture was prepared from a margarine sample (containing partially hydrogenated soybean oil) using AOCS method Ce 2-66 (5). The final concentration of the FAME sample was 20 mg/mL as specified by AOCS method Ce 1h-05 (4). No 21:0 internal standard (IS) was added to the margarine sample, as 21:0 elution was not of concern for some of this work. Some of the samples did contain 13:0 IS, though. A system suitability mixture (12:0, 9*c*-18:1, 11*c*-18:1, 11*c*-20:1, 9*c*,12*c*,15*c*-18:3, and 21:0), however, did contain 21:0 FAME. The FAME were analyzed on both CP-Sil 88 and SP-2560 columns (100 m × 0.25 mm × 0.20 μm) using an Agilent Model 6850 gas chromatograph equipped with an FID (Agilent Technologies, Little Falls, PA). The temperature program was isothermal at 180°C (3,4). Ultra high purity hydrogen (H_2) and helium (He) were the carrier gases evaluated and were run at constant flow rates of 0.6, 0.8, and 1.0 mL/min for hydrogen and 1.0 mL/min for helium. The injection inlet liner was a base-deactivated 4-mm i.d. split with glass wool (P/N 20782-211.5, Restek Corporation; P/N 5183-4647, Agilent; or P/N 092219, SGE) (4). The injector and detector temperatures were 250°C (3,4). The split ratio was approximately 100:1 (4).

RESULTS AND DISCUSSION

During the meeting in December 2003 at AOCS headquarters, the invited experts decided that the proposed chromatography

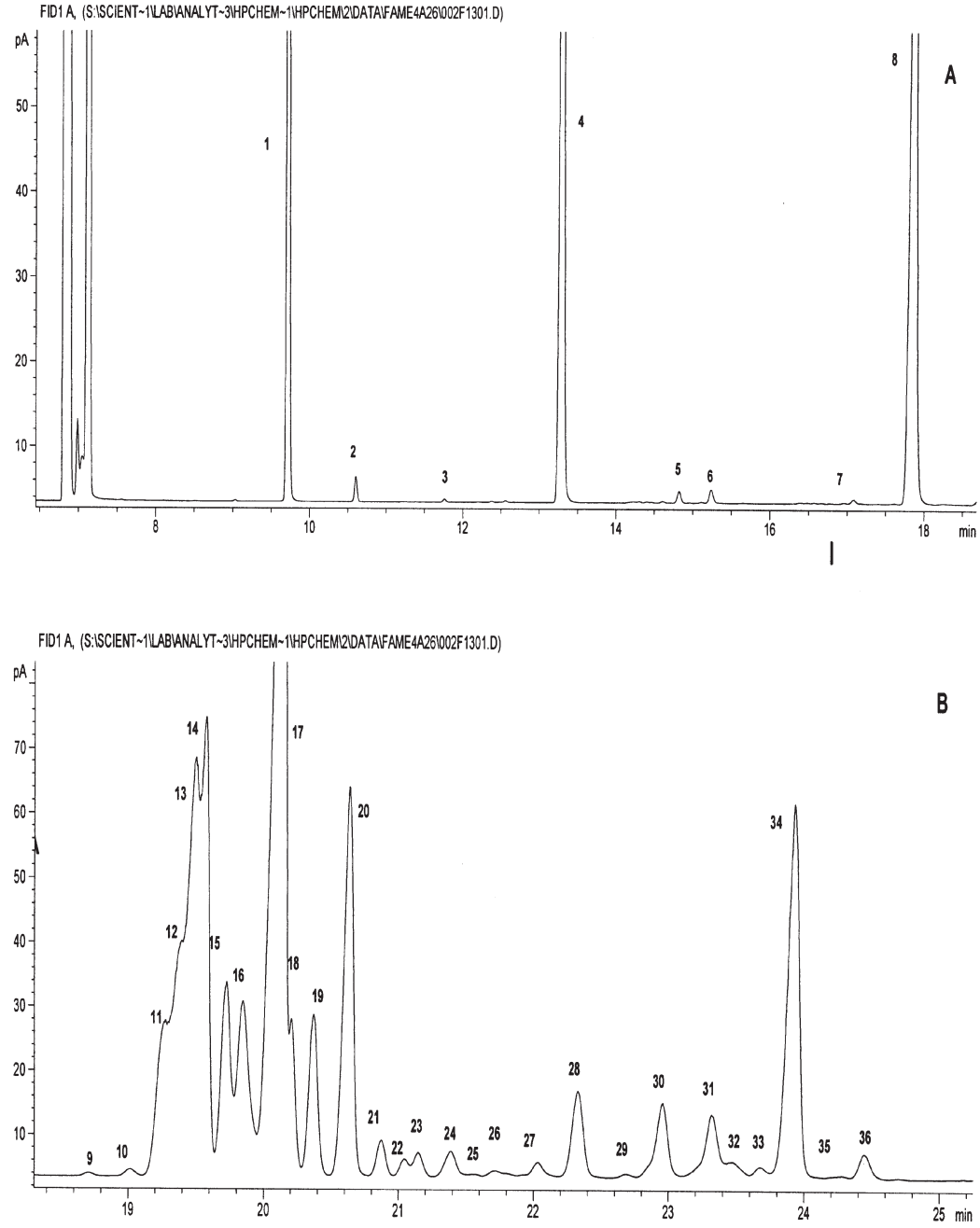

FIG. 1. Chromatogram (A = Peaks 1–8, early-eluting peaks 18:0 and less; B = Peaks 9–36, *cis*- and *trans*-18:1 and 18:2; and C = Peaks 37–50, 20:0 and later-eluting peaks) of margarine at 1.0 mL/min flow rate with hydrogen as the carrier gas at 180°C isothermal on a CP-Sil 88 column. Identifications are listed in Table 1. (Continued on next page)

for the "Determination of *cis*-, *trans*-, Saturated, Monounsaturated and Polyunsaturated Fatty Acids in Vegetable or Non-ruminant Animal Oils and Fats by Capillary GLC" method would be based on the work by Ratnayake *et al*. (3). They claimed that the use of hydrogen (H_2) as the carrier gas under isothermal conditions of 180°C and a flow rate of 0.75 mL/min offered the best separation of (i) 13*t*- + 14*t*-18:1 and 9*c*-18:1, (ii) 16*t*-18:1 and 14*c*-18:1, and (iii) 11*c*-20:1 and 9*c*,12*c*,15*c*-18:3 (α-linolenic) on the SP-2560 column. The chromatographic parameters to be used in development of the official method were agreed on by the *ad hoc* committee members at the meeting. Also recommended was the use of triheneicosanoin (21:0 TAG) as IS. This was a change from tritridecanoin (13:0 TAG), which is the IS recommended in the AOAC Official Method 996.06 for the analysis of fat content and FA composition of foods (6). Triheneicosanoin was chosen because its theoretical correction factor (TCF = 1.000) was similar to the C_{18} FA family (ranging from TCF = 1.0017 to 1.0225), as opposed to tritridecanoin

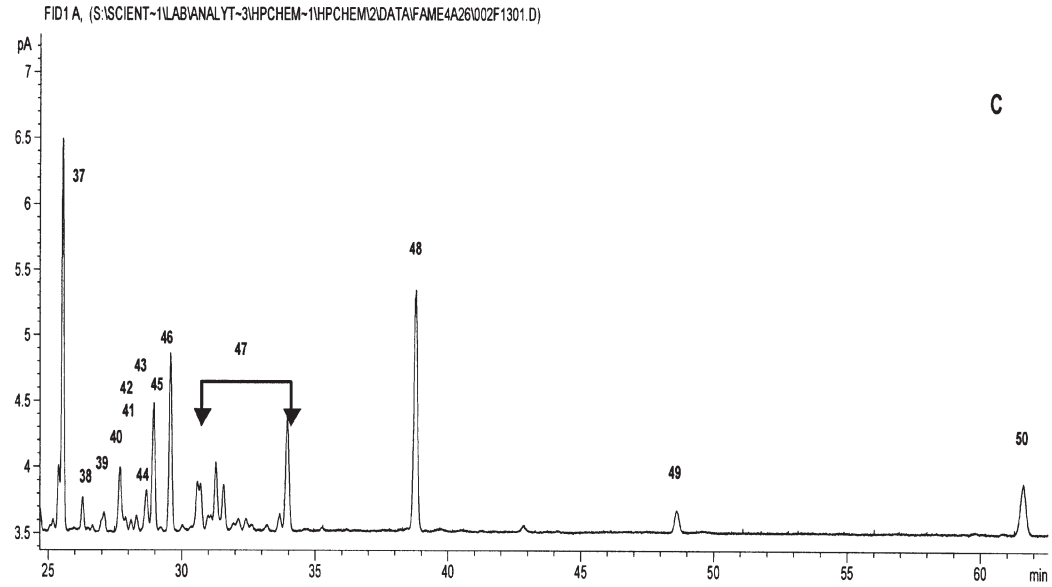

FIG. 1 (continued)

(TCF = 1.0831). There was also concern that there could be split discrimination (i.e., more volatile compounds are lost through the split port thereby lowering the actual amount of the compound that gets onto the column compared with less volatile compounds) between $C_{13:0}$ FAME and the C_{18} FAME family, because of the higher volatility of 13:0 FAME, and that the use of 21:0 would minimize this discrimination. However, since triheneicosanoin eluted in a different part of the chromatogram, it also needed to be evaluated further. It was evaluated as part of the system suitability mix, and not as part of the margarine, as the resolution of the FA naturally occurring in the margarine was examined under the proposed chromatographic parameters. It is also well known that 21:0 FAME elutes in the conjugated linoleic acid (CLA) region. However, since the regulatory

TABLE 1
Peak Identification[a] of Margarine Sample in Figure 1

Peak number	FA	Peak number	FA
1	13:0 (internal standard)	26	*tt*-NMID 18:2
2	14:0	27	9*t*,12*t*-18:2
3	15:0	28	(9*c*,13*t* + 8*t*,12*c*)-18:2
4	16:0	29	*c*,*t*/*t*,*c*-18:2
5	9*c*-16:1	30	9*c*,12*t*-18:2
6	17:0	31	9*t*,12*c*-18:2
7	10*c*-17:1	32	(9*t*,15*c* + 10*t*,15*c*)-18:2
8	18:0	33	9*c*,13*c*-18:2
9	4*t*-18:1	34	9*c*,12*c*-18:2
10	5*t*-18:1	35	9*c*,14*c*-18:2
11	(6*t*–8*t*)-18:1	36	9*c*,15*c*-18:2
12	9*t*-18:1	37	20:0
13	10*t*-18:1	38	Unknown
14	11*t*-18:1	39	9*t*,12*t*,15*c*-18:3 + 9*t*,12*c*,15*t*-18:3
15	12*t*-18:1	40	9*c*,12*t*,15*t*-18:3 + 9*c*,12*c*,15*t*-18:3
16	(13*t* + 14*t*)-18:1 + (6*c*–8*c*)-18:1	41	Unknown
17	9*c*-18:1	42	Unknown
18	10*c*-18:1 + 15*t*-18:1	43	Unknown
19	11*c*-18:1	44	9*c*,12*t*,15*c*-18:3
20	12*c*-18:1	45	11*c*-20:1 + 9*t*,12*c*,15*c*-18:3
21	13*c*-18:1	46	9*c*,12*c*,15*c*-18:3
22	16*t*-18:1	47	CLA
23	14*c*-18:1	48	22:0
24	15*c*-18:1	49	23:0
25	*tt*-NMID 18:2	50	24:0

[a]NMID, non-methylene-interrupted diene.

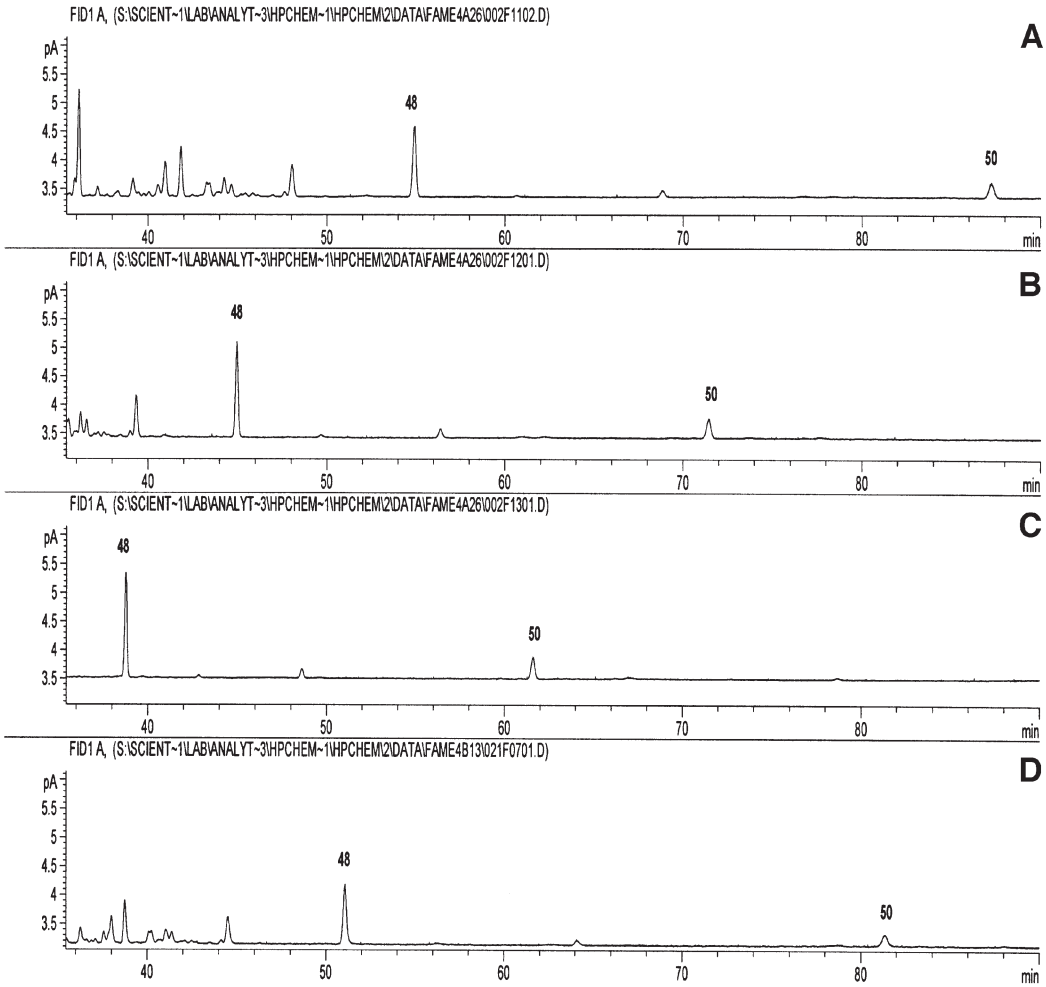

FIG. 2. Chromatograms of 24:0 FAME (methyl lignoceric) from margarine at 0.6 (A), 0.8 (B), and 1.0 (C) mL/min flow rates with hydrogen as the carrier gas at 180°C isothermal on a CP-Sil 88 column. Helium was run at 1.0 mL/min (D). Identifications are listed in Table 1.

chemical definition of the FDA (1) and Health Canada (2) for *trans* FA would exclude CLA (see "Scope of AOCS Ce 1h-05") (4) and since the concentration of CLA in most vegetable oils is low (<0.5%), 21:0 was not added to the margarine for chromatographic evaluation. Nevertheless, it should be noted here that small amounts of CLA are produced during hydrogenation and deodorization of vegetable oils (7).

For this study, a margarine oil sample containing 34.2% total *trans,* 27.9% 18:1 *trans*, 6.1% 18:2 *trans*, and 0.2% 18:3 *trans* was chosen to evaluate the optimal chromatographic conditions for optimal separation of the various *cis* and *trans* FA isomers. The chromatographic profile of the margarine is shown in Figure 1. Peaks were identified by comparing the FA profile with that reported by Ratnayake *et al*. (3) for margarine samples and also by using a standard FAME mixture (GLC-463; Nu-Chek-Prep, Inc., Elysian, MN). Quantification was on a normalized area percent basis. This chromatography was from a CP-Sil 88 column operated isothermally at 180°C. The chromatogram was divided into three regions, the early-eluting peaks less than 18:0 (A), *cis*- and *trans*-18:1 and 18:2 (B), and the later-eluting peaks greater than 20:0 (C). The peak identifications are listed in Table 1. This chromatogram serves as peak identification for subsequent analysis. Figures 2–5 show the chromatography of this margarine sample using different flow rates (0.6, 0.8, and 1.0 mL/min) and different carrier gases (H_2 and He) on a CP-Sil column. Figures 6 and 7 show the corresponding chromatography of the same margarine sample on an SP-2560 column.

Though a minor dietary FA, lignoceric acid (24:0) is present in almost all vegetable oils and animal fats and it is the FA with the highest number of carbon atoms compared with all the other common dietary FA. Because of its long carbon chain, the 24:0 FAME is very often one of the last components to elute on polar capillary GC columns. Thus, so as to cut down on the total time for GC analysis of the FA profile of fats, it is imperative that 24:0 FAME elute within a reasonable time frame. Figure 2 shows the effect of hydrogen carrier gas flow rates of 0.6, 0.8, and 1.0 mL/min on the elution time of 24:0 FAME (Peak 50) on a CP-Sil 88 (100 m × 0.25 mm × 0.20 μm) column operated isothermally at 180°C. The linear velocities were 19, 23,

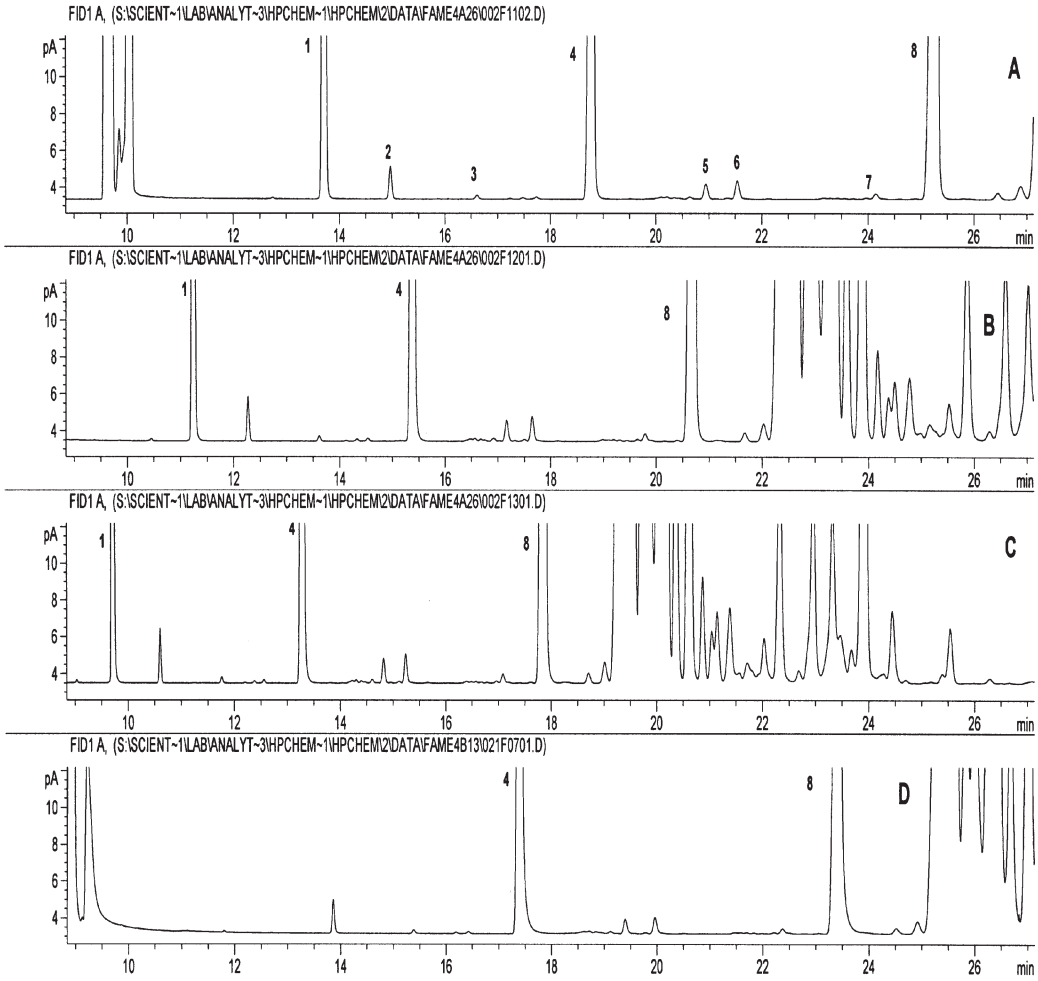

FIG. 3. Chromatograms of early-eluting margarine peaks at 0.6 (A), 0.8 (B), and 1.0 (C) mL/min flow rates with hydrogen as the carrier gas at 180°C isothermal on a CP-Sil 88 column. Helium was run at 1.0 mL/min also (D). This is the early part of the chromatograms from Figure 1. Identifications are listed in Table 1.

and 26 cm/s, respectively. At a flow rate of 1.0 mL/min, 24:0 FAME eluted approximately 20–30 min earlier compared with the 0.6 mL/min flow rate and approximately 10 min earlier compared with 0.8 mL/min. This time-saving could be a benefit to edible oil refinery laboratories where run time is very important. When the linear velocities were the same (19 cm/s), 24:0 FAME eluted at about 80–85 min for both H_2 and He carrier gases.

Figure 3 shows the early portion of the chromatograms (Peaks 1–8) presented in Figure 2. There does not appear to be any loss of resolution between 0.6, 0.8, and 1.0 mL/min in the early-eluting peaks for either H_2 or He (1.0 mL/min), suggesting a flow rate of 1.0 mL/min is the most suitable carrier gas flow rate to use at 180°C isothermal operation for the analysis of FA profiles of vegetable and animal fats and oils.

Figure 4 shows the 18:1 *cis* and *trans* FA region of the margarine chromatograms at different flow rates on a CP-Sil 88 column. The best separation was achieved with a linear velocity of 26 cm/s at 1.0 mL/min for H_2. At this flow rate, there was an improved resolution between 9*c*-18:1 (Peak 17) and 10*c*-18:1 (Peak 18) and also between 16*t*-18:1 (Peak 22) and 14*c*-18:1 (Peak 23). Comparison between H_2 (Fig. 4C) and He (Fig. 4D) as carrier gas at a flow rate of 1.0 mL/min shows that both gave poor resolution of the two pairs of isomers just mentioned.

Figure 5 shows the chromatography in the 11*c*-20:1, *cis*- and *trans*-18:3, and CLA regions, even though CLA are not a focus of this work nor are they determined with AOCS method Ce 1h-05 (4). Compared with the analyses at carrier gas flow rates of 0.6 and 0.8 mL/min, the analysis at 1.0 mL/min provided the most satisfactory separation of the different FA in this region. In particular, the four *trans*-geometrical isomers of 18:3 (i.e., 9*t*,12*c*,15*t*-18:3, 9*c*,12*c*,15*t*-18:3, 9*c*,12*t*,15*c*-18:3, and 9*t*,12*c*,15*c*-18:3) that are very often present in refined canola and soybean oils are well separated from each other. However, 11*c*-20:1 overlapped with the 9*t*,12*c*,15*c*-18:3 isomer (Peak 45; Fig. 5C) when using H_2 on a CP-Sil 88 column. Helium started to resolve this separation (Fig. 5D) but not as well as the complete baseline resolution achieved on an SP-2560 column with either H_2 or He (3). Special attention should be paid to this separation on all columns. In the laboratory of one of the authors

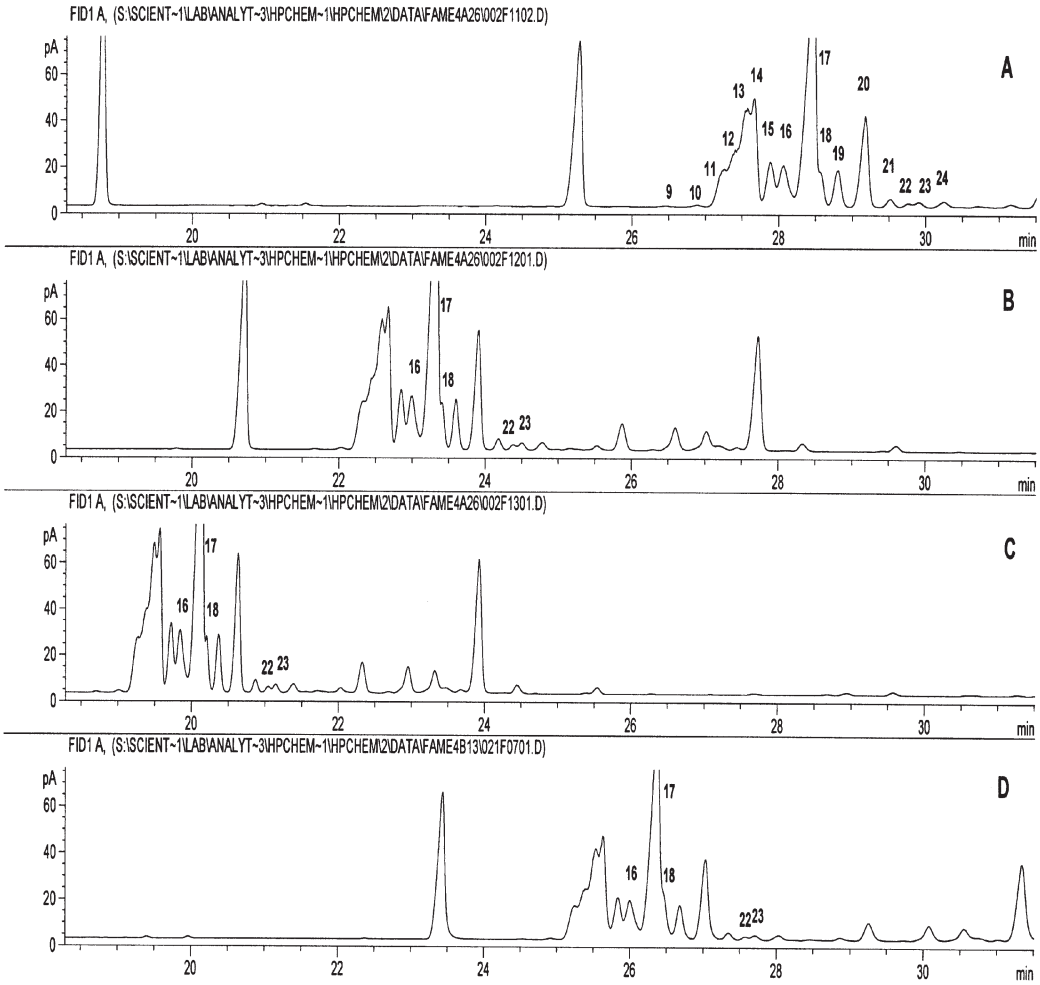

FIG. 4. Chromatograms of margarine *cis*- and *trans*-18:1 FA isomers at 0.6 (A), 0.8 (B), and 1.0 (C) mL/min flow rates with hydrogen as the carrier gas at 180°C isothermal on a CP-Sil 88 column. Helium was run at 1.0 mL/min also (D). Identifications are listed in Table 1.

(Hansen), these compounds are easily resolved on a CP-Sil 88 column when a temperature program is used in that region of the chromatogram (isothermal through the *cis*- and *trans*-18:1 and 18:2 regions); however, as shown by Ratnayake *et al*. (3), a temperature program used throughout the chromatographic run gives a less satisfactory separation of some of the *cis*- and *trans*-18:1 isomers, whereas isothermal conditions provide much improved separation of almost all the *cis*- and *trans*-18:1 isomers.

From Figures 2–5, it is clear that increasing the linear velocity from 0.6 to 1.0 mL/min reduced the run time required to elute 24:0 FAME. In addition, it improved the resolution between critical pairs. Furthermore, these chromatograms show that, compared with He, the use of H_2 as the carrier gas also facilitated the reduction in total run time without affecting the resolution between the various isomers. However, it should be noted that He was only investigated at the optimal H_2 flow rate, in an effort to harmonize the method (e.g., use the same flow rate no matter which column or carrier gas was used).

Next, a similar set of experiments was conducted on a 100-m SP-2560 capillary column. As shown in Figure 6, as observed for the CP-Sil 88 column, a flow rate of 1.0 mL/min and linear velocity of 26 cm/s also resulted in the best separation for the margarine sample. Compared with the CP-Sil 88 column, the SP-2560 column improved the resolution between 16*t*-18:1 and 14*c*-18:1 but decreased it between 9*c*-18:1 and 10*c*-18:1 for all flow rates examined. Peaks eluted on the SP-2560 about 0.5 min earlier than on the CP-Sil 88 column. The resolution between 13*t* + 14*t*-18:1 and 9*c*-18:1 was better for the CP-Sil 88 column using He as the carrier gas (Figs. 4D, 6D) as evidenced between the larger valley between Peaks 16 and 17. This is the typical separation point for *cis*- and *trans*-18:1 FA, the major isomers in hydrogenated vegetable oil samples.

In Figure 7, there did not appear to be improved resolution between the 18:3-*cis* and -*trans* isomers for the three velocities examined or between H_2 and He gases on the SP-2560 column. Compared with the CP-Sil 88, the SP-2560 resolution is still better in this region using isothermal conditions at 180°C.

In a comparison of the CP-Sil 88 and SP-2560 columns, CP-Sil 88 showed a better resolution and response between the 9*c*-

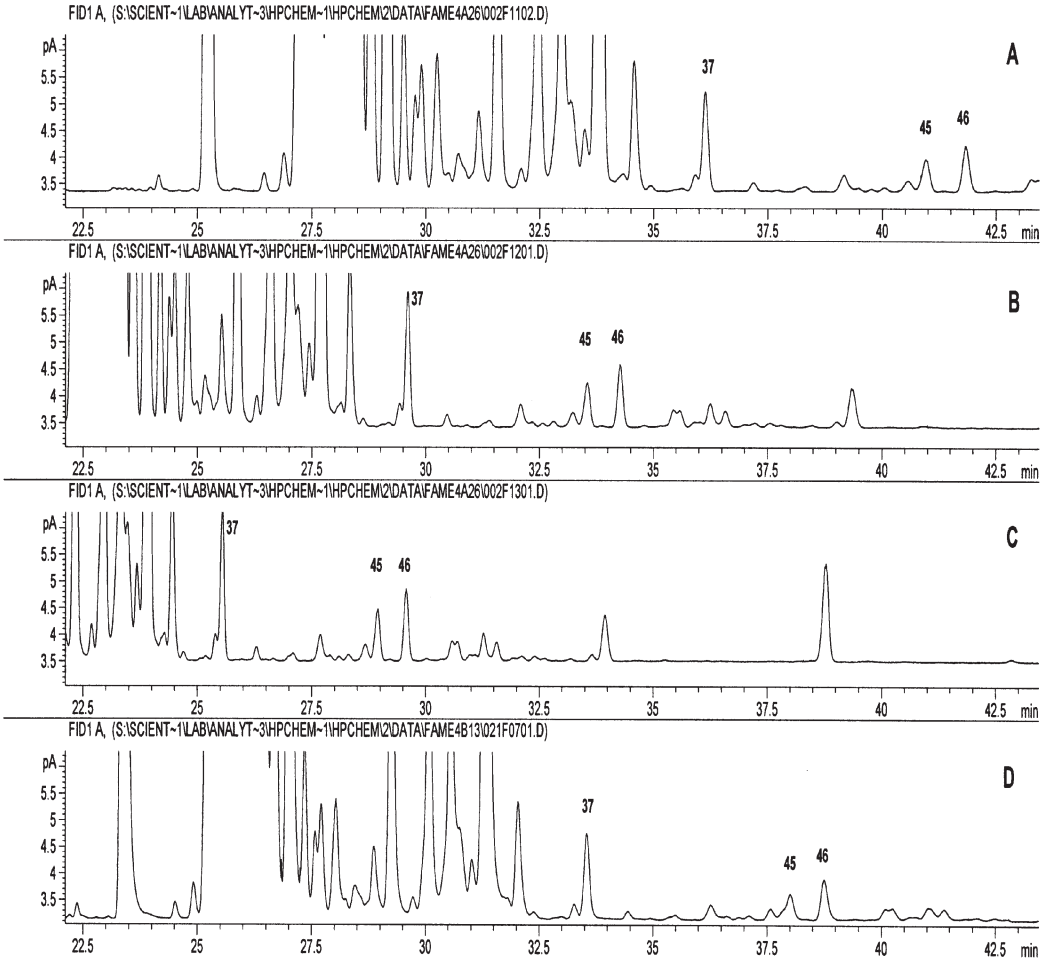

FIG. 5. Chromatograms of margarine *cis*- and *trans*-18:3, 11*c*-20:1, and CLA and regions at 0.6 (A), 0.8 (B), and 1.0 (C) mL/min flow rates with hydrogen as the carrier gas at 180°C isothermal on a CP-Sil 88 column. Helium was run at 1.0 mL/min also (D). Identifications are listed in Table 1.

18:1 and 10*c*-18:1 FA isomers using both H_2 and He carrier gases at the same flow rate (1.0 mL/min) (Fig. 8). This is not meant to imply that all CP-Sil 88 columns will always resolve these peaks better, but that they did for these columns. Methyl arachidic (20:0) appeared to be better resolved on an SP-2560 column when using He vs. the CP-Sil 88 column in Figure 9. There also appeared to be co-elution of the 18:3 *trans* isomers and 11*c*-20:1 (methyl gondoic) on the CP-Sil 88 column. This separation was not a problem on the SP-2560 column (Figs. 9B, 9D). Work by Ratnayake *et al*. (3) first showed these separation issues. The use of He started to resolve these peaks (Peak 45) on the CP-Sil 88 compared with H_2 (Fig. 9C).

Therefore, low *trans*-FA oil containing 7.2% total *trans* (5.1% 18:1 *trans*, 1.4% 18:2 *trans*, and 0.8% 18:3 *trans*) oil was analyzed on both columns (Fig. 10). This sample was chosen because of the 18:3 *trans* isomers profile. Figure 10 showed that the SP-2560 column resolved the 18:3 *trans* isomers better than the CP-Sil 88 column did. On the CP-Sil 88 column, the use of He as a carrier gas almost allowed for separation of 11*c*-20:1 and 18:3 *trans* isomers (Fig. 9C). It has been our experience that there is column-to-column variability in the resolving ability noted for the CP-Sil 88 columns, especially when using temperature programming (data not shown). One would suspect the same would hold true for the SP-2560 columns. This may be an indication that precise oven control is needed when using the nonbonded phase columns and that very slight adjustment of chromatographic parameters is needed to provide for proper resolution (3). A section in AOCS method Ce 1h-05 allows for slight adjustments to achieve the optimal separation (4). Ratnayake *et al*. (3) also discussed this issue.

In brief, the use of H_2 at the same flow rate as He results in shortening the GC run time by 20 min. However, the use of He as a carrier gas in some separations allowed for the improved resolution between the isomers of interest, but the increased run times were the trade-off. The use of a CP-Sil 88 column allowed for better response and peak sharpness, especially the early-eluting 18:1 *trans* isomer peaks, compared with the SP-2560 column, although quantification was not different. A CP-Sil 88 column provided for improved resolution between the 9*c*-18:1 and 10*c*-18:1 FA isomers, whereas the SP-2560 column provided for superior resolution between 9*t*,12*c*,15*c*-18:3 and 11*c*-20:1.

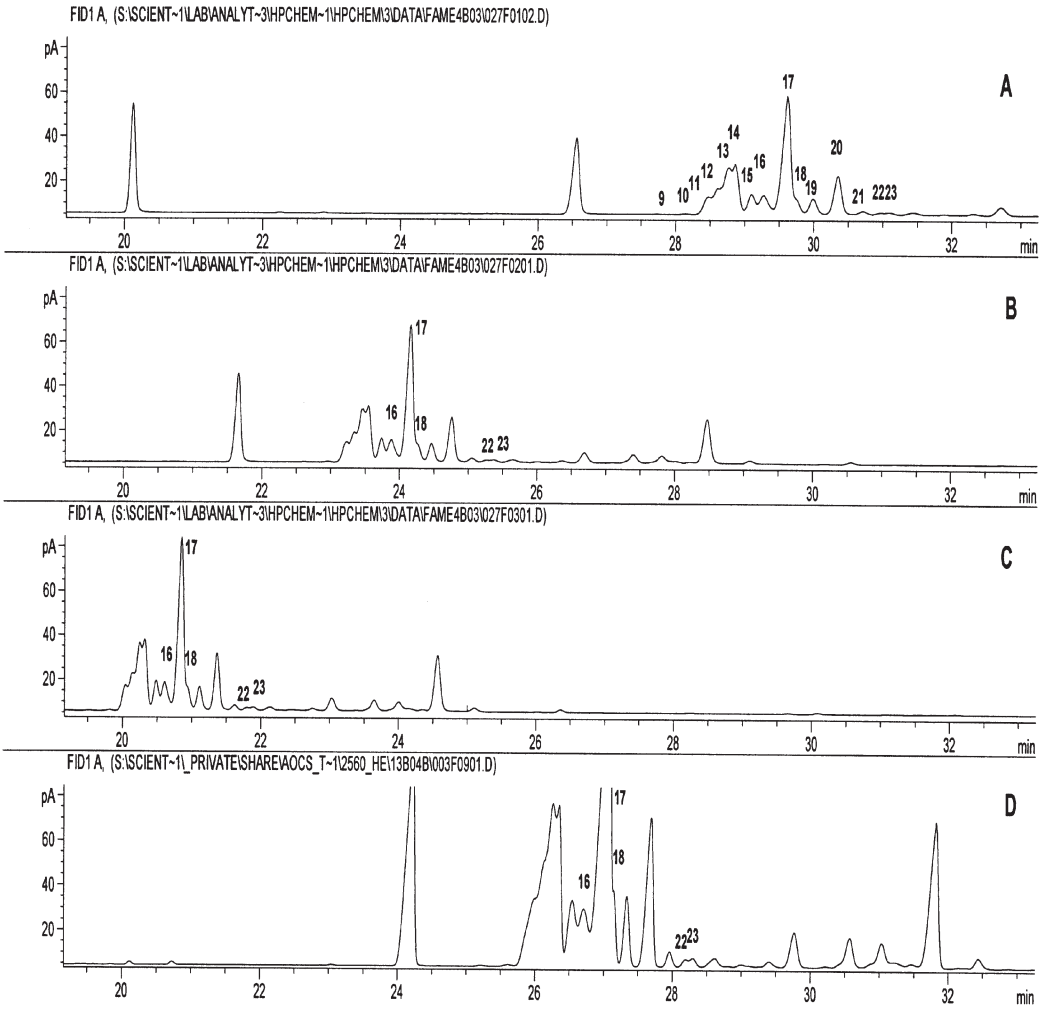

FIG. 6. Chromatograms of margarine *cis*- and *trans*-18:1 regions at 0.6 (A), 0.8 (B), and 1.0 (C) mL/min flow rates with hydrogen as the carrier gas at 180°C isothermal on an SP-2560 column. Helium was run at 1.0 mL/min also (D). Identifications are listed in Table 1.

As part of the continuing method development for Ce 1h-05, a sample mixture consisting of approximately 1 mg/mL each of 12:0, 9*c*-18:1, 11*c*-18:1, 9*c*,12*c*,15*c*-18:3, 11*c*-20:1, and 21:0 FAME in heptane was evaluated as a system suitability mixture. Figure 11 shows the chromatograms from both CP-Sil 88 and SP-2560 columns using both H_2 and He as carrier gases. Calculation of the resolution factors for the 9*c*,12*c*,15*c*-18:3 and 11*c*-20:1 separations was 2.3 and 1.9 for the CP-Sil 88 column with H_2 and He as the carrier gases, respectively (Table 2). The resolution factor for the SP-2560 column was 1.3 and 1.0 for H_2 and He, respectively (Table 2). This indicated that the CP-Sil 88 column provided a more complete baseline separation than the SP-2560 did. Therefore, it would be expected that 11*c*-20:1 would be better resolved from the *trans*-18:3 isomers; however, as mentioned earlier, this is not the case, as it appears that the 18:3 *trans* isomers elute independently of each other and of the *cis* isomer. This improved resolution actually caused an interference with 9*t*,12*c*,15*c*-18:3, suggesting that resolution factors may not be required in this method or that different standards should be used in the system suitability mixture. Although the CP-Sil 88 column actually does provide for a much better resolution between 11*c*-20:1 and 9*c*,12*c*,15*c*-18:3, the 18:3 *trans* isomers are not as well resolved, and therefore, this suitability mixture could be misleading for determining which column is best for resolution of *cis*- and *trans*-18:3 isomers. This separation also confirmed that certain isomers (e.g., 18:3) may elute independently of other isomers, and care must be exercised when determining a suitable system suitability mixture. Hydrogen also allowed for better resolution than He between 11*c*-20:1 and 9*c*,12*c*,15*c*-18:3; again this was not beneficial for the 18:3 *trans* isomers. It appears that a resolution factor between 11*c*-20:1 and 9*c*,12*c*,15*c*-18:3 of 1.0–1.5 would be optimal.

Since resolution of 16*t*-18:1 and 14*c*-8:1 in margarine is a key separation in determining the resolving ability of the GC columns and since standards of these isomers are not readily available, it was advised that margarine should be used to calculate the response factors. However, resolution factors were

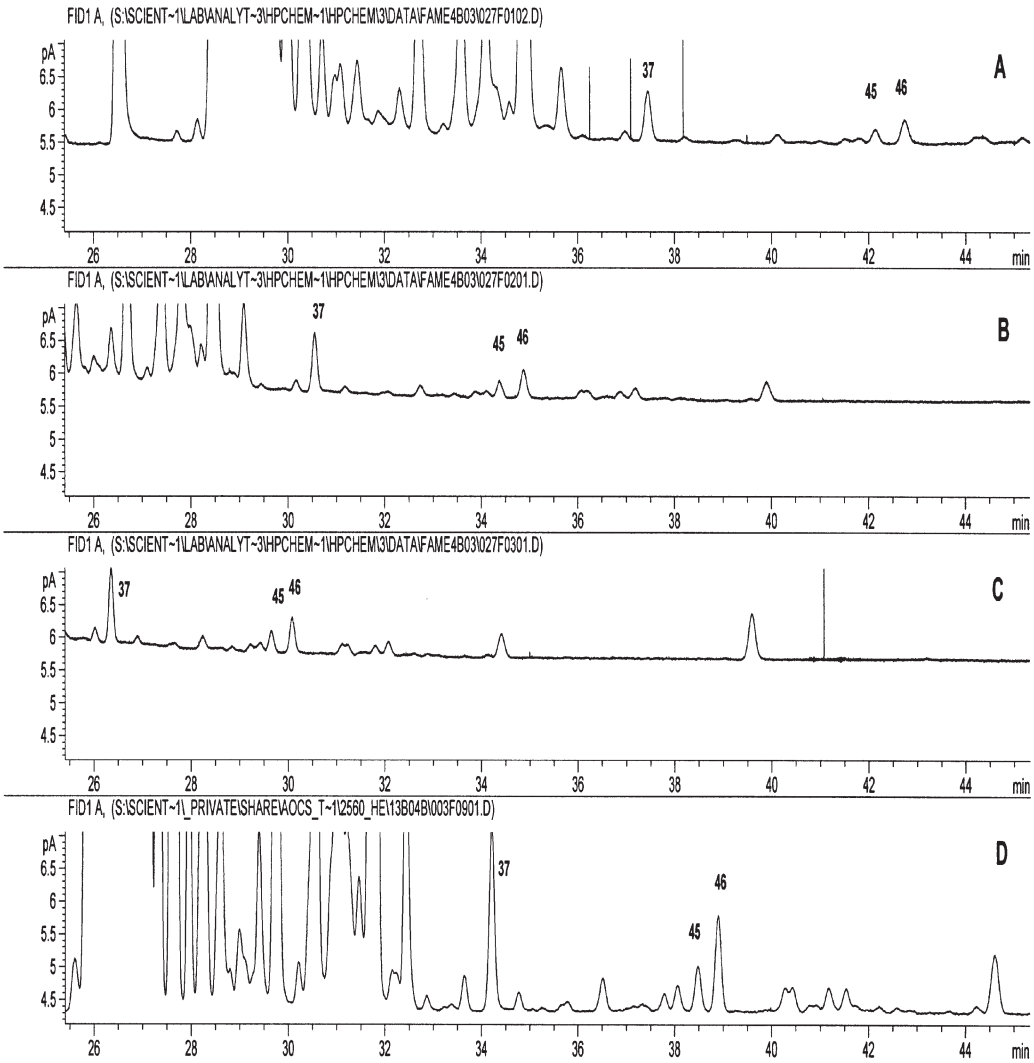

FIG. 7. Chromatogram of margarine *cis*- and *trans*-18:3, 11*c*-20:1, and CLA regions at 0.6 (A), 0.8 (B), and 1.0 (C) mL/min flow rates with hydrogen as the carrier gas at 180°C isothermal on an SP-2560 column. Helium was run at 1.0 mL/min also (D). Identifications are listed in Table 1.

TABLE 2
Calculation of Resolution Factors with Different Carrier Gases (H_2 and He) at a Flow Rate of 1.0 mL/min at 180°C Isothermal on Both the CP-Sil 88 and SP-2560 Columns

Resolution factors	CP-Sil 88 H_2	CP-Sil 88 He	SP-2560 H_2	SP-2560 He
C18:1, 13*t* and C18:1, 9*c* Margarine	1.3	ND[a]	1.1	1.0
C18:1, 9*c* and C18:1, 11*c* standards	2.0	2.0	2.4	2.3
C18:1, 16*t* and C18:1, 14*c* margarine	0.5	ND	ND	0.3
C20:1, 11*c* and C18:3, 9*c*,12*c*,15*c* standards	2.3	1.9	1.3	1.0

[a]ND, not determined.

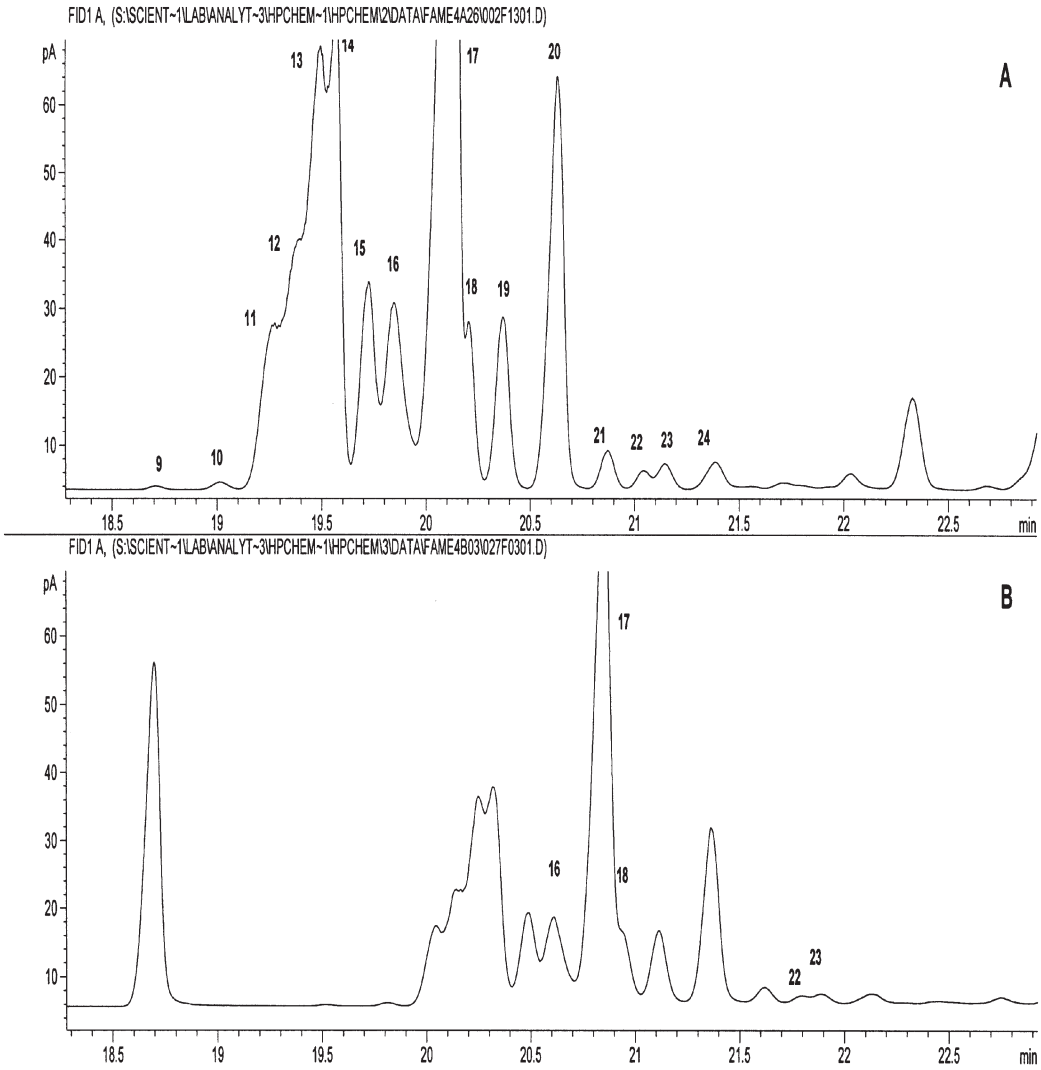

FIG. 8. Chromatograms of the *cis*- and *trans*-18:1 regions of margarine at 1.0 mL/min flow rates with hydrogen as the carrier gas at 180°C isothermal on a CP-Sil 88 (A) and SP-2560 (B) column, respectively. Identifications are listed in Table 1.

difficult to determine accurately for these compounds and therefore, a visual observation of this separation coupled with calculation of 9*c*-18:1 and 11*c*-18:1 and of 11*c*-20:1 and 9*c*,12*c*,15*c*-18:3 resolution factors should be sufficient for determining the resolving power of the column necessary for the determination of *cis* and *trans* isomers.

The 21:0 FA, which will be used as the IS in AOCS Official Method Ce 1h-05, appeared to be overloaded at 1 mg/mL on both columns and with both carrier gases as evidenced by the tailing factor calculation (ranging from 0.29 to 0.48) (Table 3).

The amounts of 12:0 and 21:0 methyl esters detected on the CP-Sil 88 column were very similar when compared with the SP-2560 column depending on conditions as shown in Table 3. The amounts were calculated by dividing the sample amount (mg), dissolved in an exact volume of solvent, by the response amount (pA) and then dividing by the TCF. These data indicate that split discrimination would not be a problem. The 12:0 peaks appeared to be very Gaussian on both columns and with both carrier gases (ranging from 0.97 to 1.14) (Table 3). The use of 21:0 TAG as the IS was thought to be beneficial because its TCF is closer to the C_{18} FA family TCF values and because there may be split discrimination of the short- and medium-chain FA.

Table 4 shows the normalized area percentage concentrations for total *trans*, *trans*-18:1, *trans*-18:2, *trans*-18:3, CLA, 16:0, and 18:0 of the margarine sample when analyzed on different columns with different carrier gases and flow rates. All the values appear to be very similar in composition. Even though there was slight co-elution between *trans*-18:3 and 11*c*-20:1 when H_2 was used as the carrier gas as compared with He, quantification differences were minimal. However, quantification differences will depend on the amount of *trans*-18:3 isomers present in a sample.

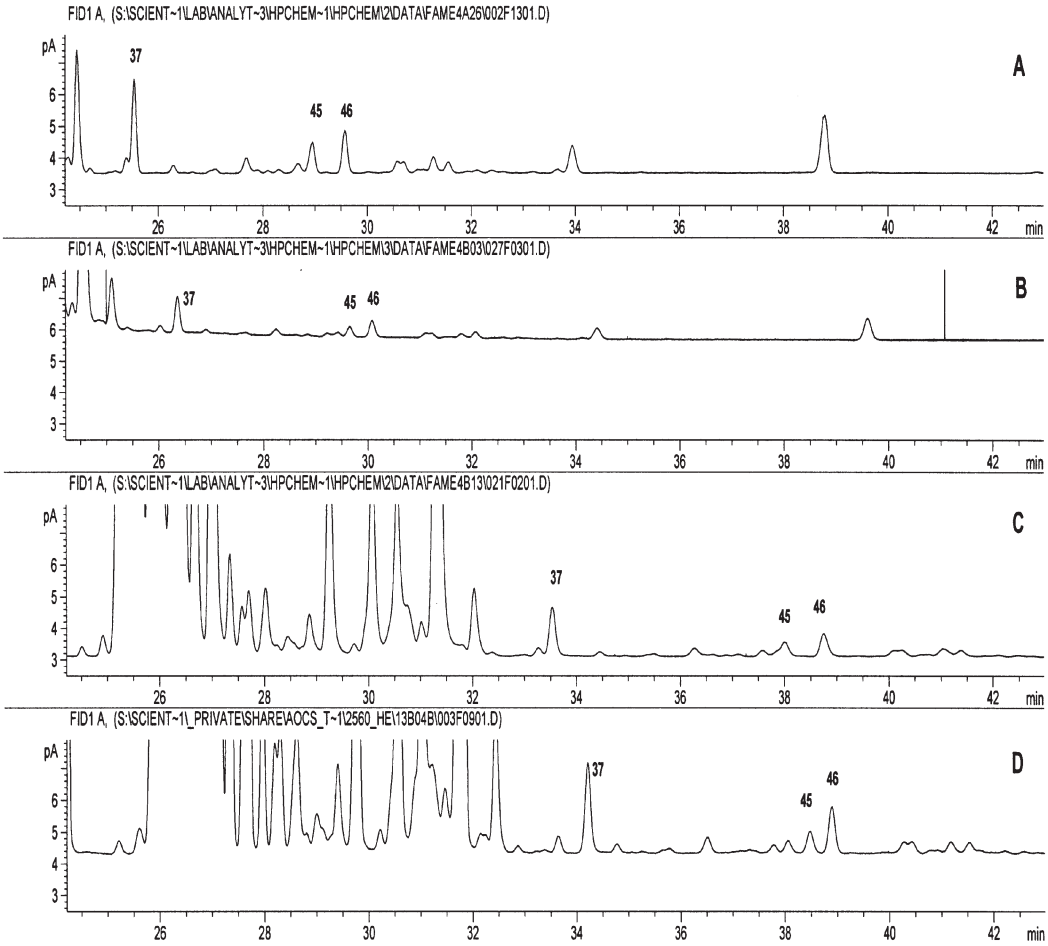

FIG. 9. Chromatograms of 20:0, 11*c*-20:1, and 9*c*,12*c*,15*c*-18:3 from margarine at 1.0 mL/min flow rates with hydrogen as the carrier gas at 180°C isothermal on a CP-Sil 88 (A) and SP-2560 (B) column, and with helium as the carrier gas at 180°C isothermal on a CP-Sil 88 (C) and SP-2560 (D) column. Identifications are listed in Table 1.

TABLE 3
Calculation of Tailing Factors and Amounts with Different Carrier Gases (H_2 and He) at a Flow Rate of 1.0 mL/min at 180°C Isothermal on Both the CP-Sil 88 and SP-2560 Columns

Response/ discrimination	CP-Sil 88 H_2 12:0	CP-Sil 88 H_2 21:0	CP-Sil 88 He 12:0	CP-Sil 88 He 21:0	SP-2560 H_2 12:0	SP-2560 H_2 21:0	SP-2560 He 12:0	SP-2560 He 21:0
Tailing factor	1.00	0.38	1.14	0.48	1.00	0.47	0.97	0.29
Amount	48.4	49.6	48.4	49.6	48.4	49.6	48.4	49.6
Area	174.0	197.7	210.5	240.7	189.1	181.4	293.7	273.3
Amount/area	0.2782	0.2509	0.2299	0.2061	0.2559	0.2734	0.1648	0.1815
Theor. FID correction factor	1.1013	1.0000	1.1013	1.0000	1.103	1.0000	1.1013	1.0000
Actual amounts	0.2526	0.2509	0.2088	0.2061	0.2324	0.2734	0.1496	0.1815

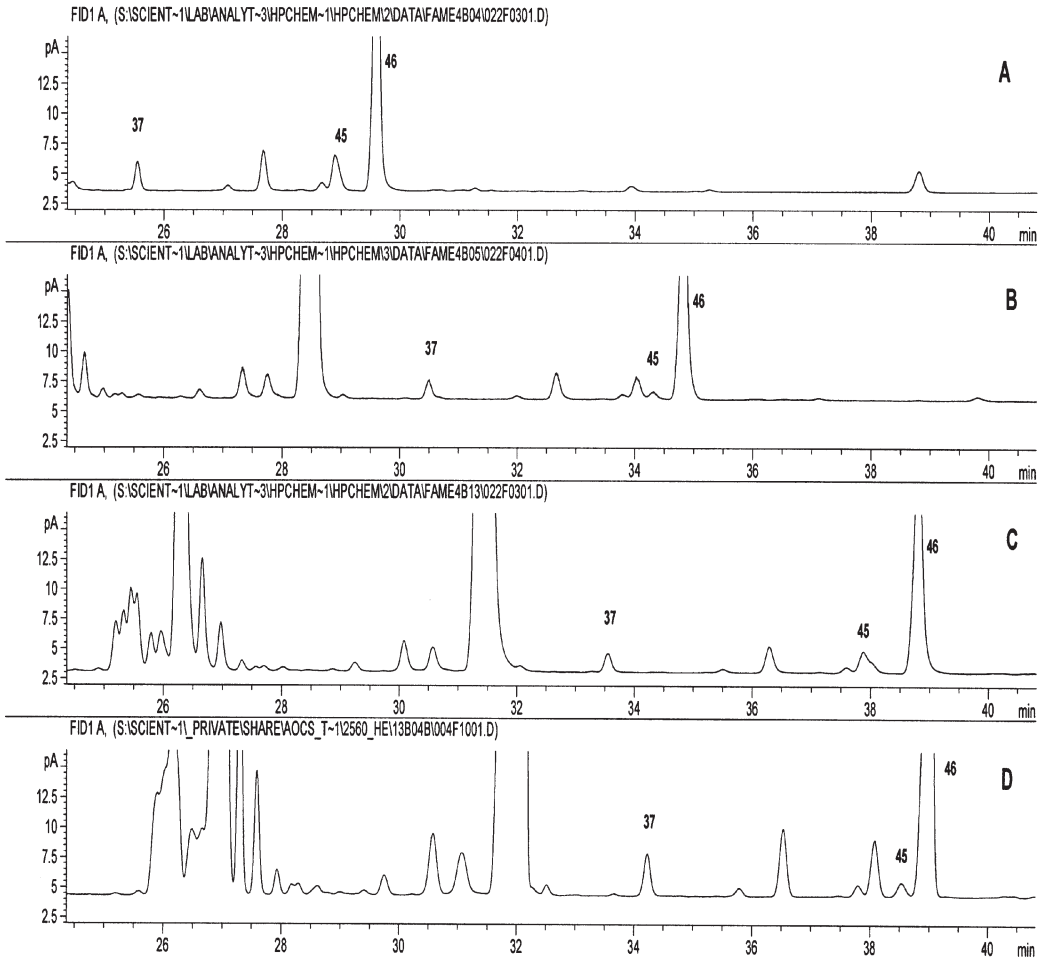

FIG. 10. Chromatogram of a low-*trans* (*ca.* 7%) sample in the 11*c*-20:1 and 9*c*,12*c*,15*c*-18:3 regions at 1.0 mL/min flow rates with hydrogen as the carrier gas at 180°C isothermal on a CP-Sil 88 (A) and SP-2560 (B) column, and with helium as the carrier gas at 180°C isothermal on a CP-Sil 88 (C) and SP-2560 (D) column. Identifications are listed in Table 1.

TABLE 4
Quantification (normalized area percentage) of the Margarine Sample at Different Linear Velocities and with Different Carrier Gases on the CP-Sil 88 and SP-2560 Columns

	0.6 mL/min CP-Sil 88 H_2	0.8 mL/min CP-Sil 88 H_2	1.0 mL/min CP-Sil 88 H_2	1.0 mL/min CP-Sil 88 He	1.0 mL/min SP-2560 H_2	1.0 mL/min SP-2560 He
Total *trans*	34.41	34.33	34.37	34.30	33.98	34.09
18:1 *trans*	28.06	28.01	28.04	27.91	27.92	27.85
18:2 *trans*	6.09	6.07	6.08	6.09	5.87	6.02
18:3 *trans*	0.26	0.25	0.25	0.30	0.19	0.22
CLA	0.55	0.51	0.56	0.54	0.32	0.41
16:0	11.79	11.76	11.78	11.65	11.91	11.96
18:0	11.52	11.51	11.51	11.08	10.74	10.98

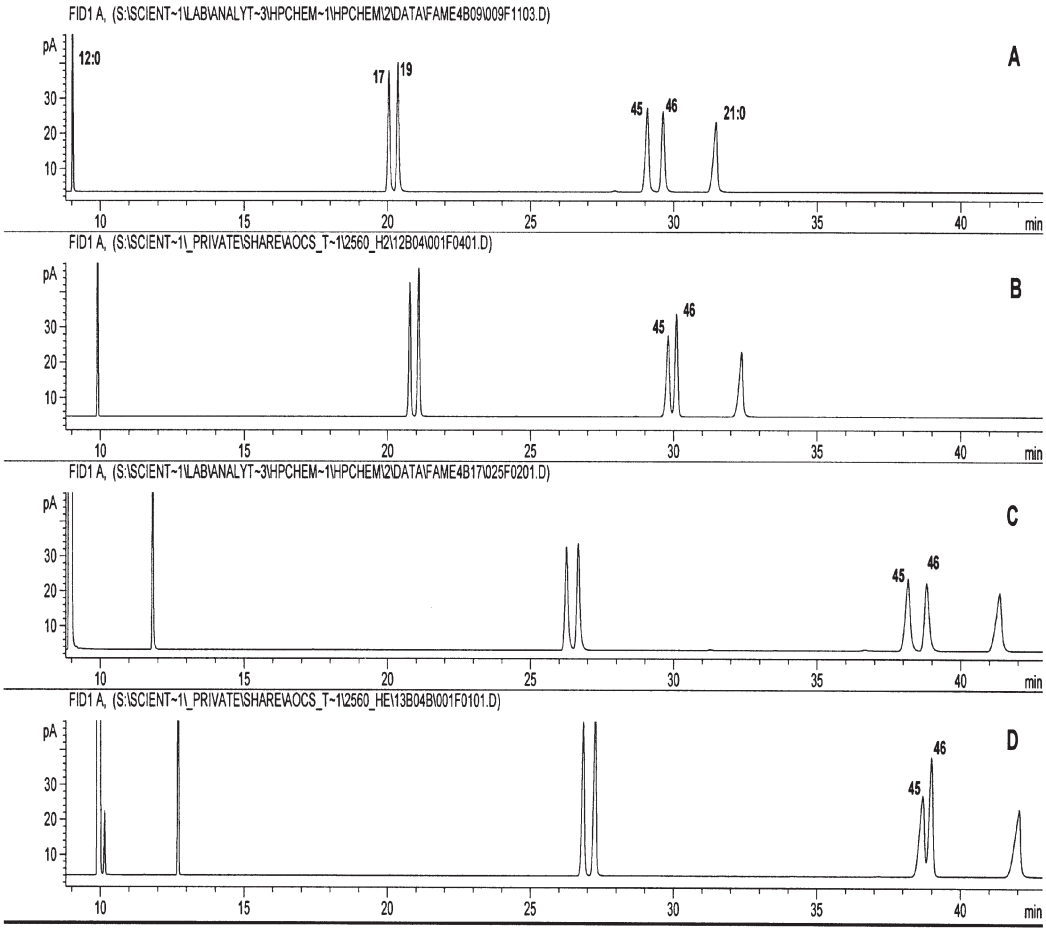

FIG. 11. Chromatogram of system suitability mixture (12:0, 9*c*-18:1, 11*c*-18:1, 11*c*-20:1, 9*c*,12*c*,15*c*-18:3, and 21:0) at 1.0 mL/min flow rates with hydrogen as the carrier gas at 180°C isothermal on a CP-Sil 88 (A) and SP-2560 (B) column, and with helium as the carrier gas at 180°C isothermal on a CP-Sil 88 (C) and SP-2560 (D) column. Identifications are listed in Table 1.

TABLE 5
Peak Sharpness of 9*c*-18:1 Peak at Different Linear Velocities and with Different Carrier Gases on the CP-Sil 88 and SP-2560 Columns

18:1 *cis*	0.6 mL/min CP-Sil 88 H_2	0.8 mL/min CP-Sil 88 H_2	1.0 mL/min CP-Sil 88 H_2	1.0 mL/min CP-Sil 88 He	1.0 mL/min SP-2560 H_2	1.0 mL/min SP-2560 He
Height (*H*)	103.4	132.3	151.1	92.8	152.0	147.3
Area (*A*)	1455	1536	1518.1	1212.9	1636	2336.4
H/A	0.071	0.086	0.0995	0.077	0.093	0.063

In Table 5, the peak sharpness was calculated by dividing the peak height (*H*) by the area (*A*), with a higher *H/A* value indicating a sharper peak for 9*c*-18:1. A flow rate of 1.0 mL/min with H_2 on both CP-Sil 88 and SP-2560 columns gave the sharpest peaks (0.0995 and 0.0930), respectively; therefore, H_2 produced the sharpest peaks. All other flow rates and carrier gases gave less than unity for the *H/A* ratio.

The foregoing indicates some of the critical separations that must be examined when using SP-2560 and CP-Sil 88 columns. One should keep in mind that there will be variability between and within columns and that one should always check on the separation before beginning analyses to determine if small adjustments must be made to the chromatographic system to ensure proper quantification and identification of *cis* and *trans* monounsaturated FA and PUFA.

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