Direct Measurement of Total *trans*- and *cis*-Octadecenoic Fatty Acids Based on a Gas–Liquid Chromatographic Class Separation of *trans*-18:1 and *cis*-18:1 Fatty Acid Methyl Esters

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Abstract

A gas–liquid chromatographic (GLC) class separation is described whereby the contents of total *trans*- and *cis*-octadecenoic fatty acids can be obtained for relatively simple samples such as margarines and for more complicated food mixtures and biological specimens. The method is an adjunct to the standard process commonly used for quantitating the general fatty acid profile of a sample (extraction of lipids, preparation of methyl ester derivatives of fatty acids, GLC separation on Carbowax-20M capillary columns). There is no incremental work beyond the addition of one chromatographic run on a cyanosilicone capillary column at 242°C that lasts only 20–25 min. The chromatograms provide a composite *trans* peak and a composite *cis* peak with sufficient resolution between them to provide direct estimation of total *trans*- and *cis*-octadecenoic fatty acids. A concurrent analysis for the general profile of fatty acids in an unknown (using Carbowax-20M columns) is included with every analysis for *trans* content to check for internal consistency and for general quality control.

Introduction

A rapid, rugged, and cost-effective method is needed for the study of *cis* and *trans* fatty acids. Such a method would be used to expand our knowledge of their levels in partially hydrogenated fats and oils; in fats associated with the meat and milk of ruminant animals; in various entrees, recipes, and menus; and in biological tissues and fluids. Reliable data on the levels of total C_{18} *trans* and *cis* monounsaturated acids (total 18:1 *trans* and *cis*) have been and continue to be of particular interest to scientists, given the responsibility of compiling and updating the compositional databases used in nutrition monitoring and other activities directed toward gaining a better understanding of diet–health relationships.

Optimally, any new method would provide insight on the contents of every one of the isomeric 18:1 acids, which would include up to 13 different *trans* members (18:1Δ*Xt*, where *X* denotes the position of the ethylenic bond with respect to the carboxyl carbon) and approximately the same number of *cis* contributors (18:1Δ*Xc*). Historically, however, due to a lack of specificity in available chemical methods, the figures most in demand have been those intended to represent simply the total 18:1 *trans* and total 18:1 *cis* content. Most often, the values reported out of the chemical laboratory that are intended to represent the level of total *trans* content have been obtained by adding the gas–liquid chromatographic (GLC) responses for as many of the peaks as can be identified. The theory is simple enough, but in practice, fairly serious difficulties have persisted. The individual isomeric C_{18} acids are never completely resolved, even on the most sophisticated capillary columns. Complications arise with the lack of uniformity in the GLC conditions, especially with the plethora of temperature programs being used in various locations. As a result, scientists must use their individual judgments in deciding which peaks to add. As a result, the ultimate consumers of the data often never know precisely what the figures represent, in part because the chromatographic traces do not normally accompany the numerical values.

The general consensus has been that GLC by itself is incapable of providing a class separation of *trans*-18:1 fatty acids from *cis*-18:1 fatty acids due to the complexity of isomer mixtures that are present. As a result, emphasis has been placed on devising ways to increase the specificity of existing GLC methods, largely by the use of ancillary techniques (for reference, see Firestone and Sheppard [1], Wolff [2], and Ratnayake [3,4]). For example, silver-ion thin-layer chromatography (Ag-TLC) is currently used as a prepurification step prior to capillary GLC (3,5–7). Likewise, there have been encouraging developments in the use of silver-ion high-performance liquid chromatography (Ag–HPLC) (8–10) in the fractionation of fatty acid methyl esters (FAMEs) according to the number and geometry of ethylenic carbon–carbon double bonds; methods will doubtlessly soon appear that use Ag–HPLC in a semipreparative mode prior to GLC. Also, the use of "correction factors" has worked well for some laboratories (6,11). A combined GLC–infrared spectrophotometric (GLC–IR) method has been developed, subjected to collaborative study, and adopted

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as a first action by AOAC International (4). Although all of these approaches have advanced the technology, none have yet filled the need for a truly rapid "routine" procedure. Present circumstances suggest a valuable role for a single-step GLC method that would directly report total *trans*- and total *cis*-C_{18}-monoenes, even if it did not provide detailed information on the multiplicity of positional isomers within these two monoene classes.

This report describes operational parameters for GLC that accomplish the long-sought-after *trans*-18:1–*cis*-18:1 class separation and do so in only 20 min. No additional steps or techniques were needed beyond those customarily used for general fatty acid analysis, which typically involves the separation and quantitation of methyl esters on commercially available capillary columns.

Experimental

The GLC was a Hewlett-Packard model 5840 with a flame-ionization detector and split–splitless capillary inlet. The injector and detector temperatures were 250 and 275°C. Helium and hydrogen carrier gases (ultrapure carrier, 99.999% [Air Products and Chemicals, Allentown, PA]) were further purified with traps of charcoal, molecular sieve, and oxygen scrubber as previously described (12). Linear velocity through the column was typically 20 cm/s (helium) or 35 cm/s (hydrogen) (adjusted while at operating temperature). Sample injections (1–4 µL) were made with a 10-µL syringe (Hamilton 7101) using a split ratio of approximately 100:1. The injection liners were deactivated inlet sleeves prepared as previously described (12). FAME elutions were catalogued and studied using three different variants of the popular cyanopropyl-type liquid phase, all applied to fused-silica open-tubular capillary columns. An SP-2380 column (poly[90%-biscyanopropyl-10%-cyanopropylphenylsiloxane], "stabilized", 0.30-µm film, 100 m × 0.20-mm i.d.) was used in "direct" GLC analysis of total *cis*- and *trans*-18:1 (e.g., Figure 2). It was a custom design prepared on special request through Mr. Len Sidisky at Supelco (Bellefonte, PA). Helium was used as the carrier gas for runs at temperatures above approximately 200°C; otherwise, hydrogen was used. An SP-2560 column (cyanopropyl substitution not specified, "nonbonded", 0.20-µm film, 100 m × 0.25-mm i.d.) was used for standard FAME separations made at column temperatures below 200°C (e.g., Figure 1), and hydrogen was used as the carrier gas. Extensive use was made of the elution data of Bannon et al. (13); their column was SP-2330 (poly[80%-biscyanopropyl-20%-cyanopropylphenylsiloxane], "nonbonded", 0.20-µm film, 60 m × 0.25-mm i.d.), and they reported using hydrogen as the carrier. These three columns, all with phases bearing the "SP" designation, were obtained from Supelco. The SP-2560 column had catalog #2-4056, and a column exactly matching the description of the one reported by Bannon et al. (13) is listed as #2-4020. FAME standards were purchased from Sigma Chemical (St. Louis, MO), Nu Chek Prep (Elysian, MN), and Matreya (Pleasant Gap, PA). A positional *cis–trans* isomer mix consisting of a margarine extract with methyl eicosanoate and methyl docosanoate was purchased from Supelco and Matreya.

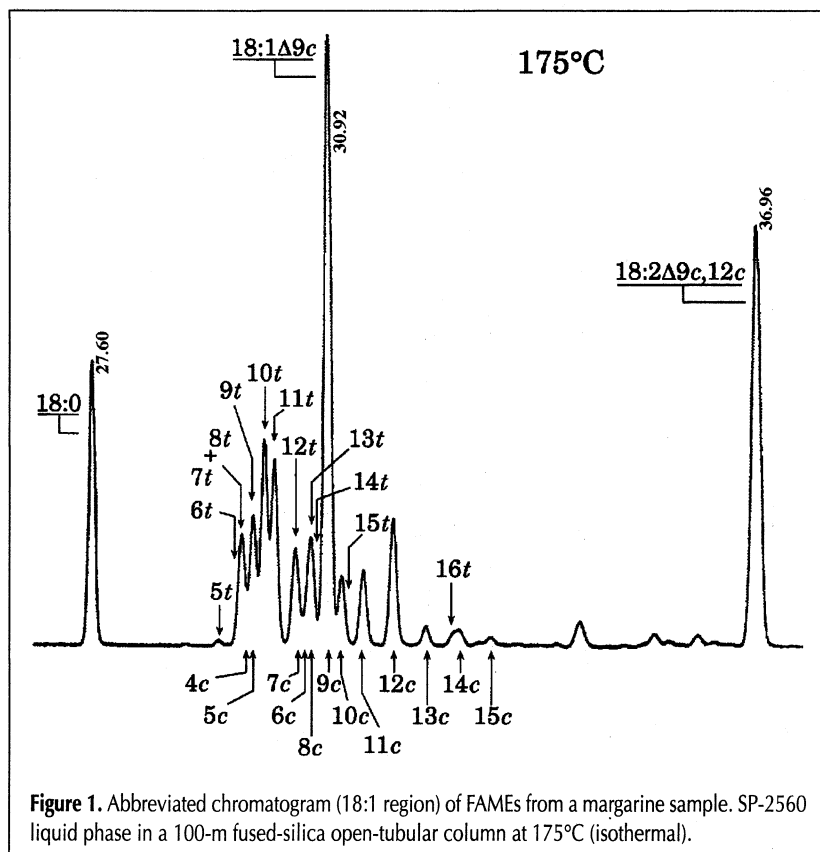

Figure 1. Abbreviated chromatogram (18:1 region) of FAMEs from a margarine sample. SP-2560 liquid phase in a 100-m fused-silica open-tubular column at 175°C (isothermal).

Results and Discussion

Traditional "direct" GLC

The "traditional" GLC approach to the analyses of total *cis*- and *trans*-18:1 fatty acids has been to carry out their separation as FAME derivatives on rather lengthy capillary columns (50–100 m) using one of the highly polar cyanosilicone-type liquid phases at column temperatures in the range of 165–185°C. The chromatogram in Figure 1, a run of FAMEs prepared from a representative margarine sample, illustrates probably the best degree of separation for 18:1 isomers that can reasonably be expected under such conditions (175°C, 100-m column length, SP-2560 phase). Arrows indicate the elution places for 24 different positional and configurational 18:1 isomers, each specified using a notation of the form *Nt* or *Nc* to indicate double-bond position (*N*) with respect to the carboxyl carbon, and either *trans* or *cis* configuration. It is not uncommon to find that laboratories with considerable expertise in *trans* fatty acid work will use a shorter column, often 50 or 60 m in length, but the

most satisfactory results in this laboratory have been with 100-m versions like the one used in Figure 1. The benefits of using the 100-m length have also been presented by Wolff and Bayard (14).

While much can be learned about the *trans*- and *cis*-18:1 contents of a sample using this "traditional" GLC separation approach, it is well-known that a complete separation of all the 18:1 isomers is too much to expect using GLC alone, regardless of how long the column is or how well it is constructed. Substantial errors may arise, depending on the extent of the overlaps that occur between the peaks for the various *cis*- and *trans*-18:1 isomers. The reader is referred to the papers of Ratnayake and colleagues (3,15–18) for comprehensive treatment of this subject, complete with details on the GLC retentions of the 18:1 and 18:2 isomers and a thorough discussion on the ramifications of overlapping peaks on the accuracy of available GLC methodologies. Bayard and Wolff (19) have also stated the problem forcefully and have reported that direct GLC gives *trans*-18:1 acid values that are 12–28% lower than their preferred method, which involves Ag–TLC prior to GLC.

Sorting out 18:1 peaks

In a typical chromatogram such as the one in Figure 1, the contributions of 18:1Δ5*t*–18:1Δ11*t* can be assessed without difficulty by considering the combined areas of the five (marginally) resolved peaks labeled 5*t*, 6*t*/7*t*/8*t*, 9*t*, 10*t*, and 11*t*. The only complicating factor is the possible presence of 18:1Δ5*c* and 18:1Δ4*c*, but there is abundant information in the literature on the relative distribution of 18:1 isomers resulting from hydrogenation to support a strategy of simply disregarding any minuscule contributions from 18:1Δ5*c* and 18:1Δ4*c*, due to their very minor contributions based both on theory and chemical analysis (3,6,11,20–27). Also in Figure 1, the reporting of 18:1Δ12*t* is confounded somewhat by 18:1Δ7*c* and to a lesser extent by 18:1Δ6*c*. The most serious difficulties are with the 18:1 *trans* acids that have high Δ values, namely 18:1Δ13*t*, 18:1Δ14*t*, and 18:1Δ15*t*; their co-location with the major *cis* isomers (18:1Δ8*c*, 18:1Δ9*c*, and 18:1Δ10*c*) cannot be disregarded, making it necessary to resort to a prepurification step, generally either Ag–TLC or Ag–HPLC, to find their level with sufficient accuracy.

Every laboratory group confronted with these analyses has doubtlessly been frustrated with this failure to fully resolve the myriad 18:1 isomers into two classes, *cis* and *trans*. Attempts have been made to improve the situation by adjusting the column's temperature, up or down, by a few degrees. Unfortunately, as experiences in the author's laboratory can attest, small changes (e.g., ± 5 or 10°C) to either side of the "standard temperature" (generally 165–185°C) only make matters worse. Also, columns longer than 100 m do not add sufficiently enough to the resolution of the isomers to justify their cost nor the additional aggravation that their manufacture and use entails. As a result, little hope has been expressed regarding the prospects for the development of a class separation that depends on GLC alone (direct GLC).

Single-step class separation of *trans*-18:1 and *cis*-18:1

The new type of chromatographic result illustrated in Figure 2 provides a much more convenient route to quantitation of (total) *trans*-18:1 and *cis*-18:1 fatty acids. All that is required to produce this result is to operate the GLC column at 242°C ± 2, some 70°C above the 175°C ± 10 temperature that heretofore has been standard operating procedure. The rationale for selecting a temperature of 242°C ± 2 is developed below.

In Figure 2, the two composite peaks, the *trans*-18:1 peak and the *cis*-18:1 peak, show a virtually complete class separation at 242°C on a 100-m column. The *trans*-18:1 peak contains all 11 of the C_{18}-monounsaturated *trans* components that are quantitatively significant in partially hydrogenated fats and oils. Likewise, the *cis*-18:1 peak contains all 10 important C_{18}-monounsaturated *cis* acids. In Table I, all of the

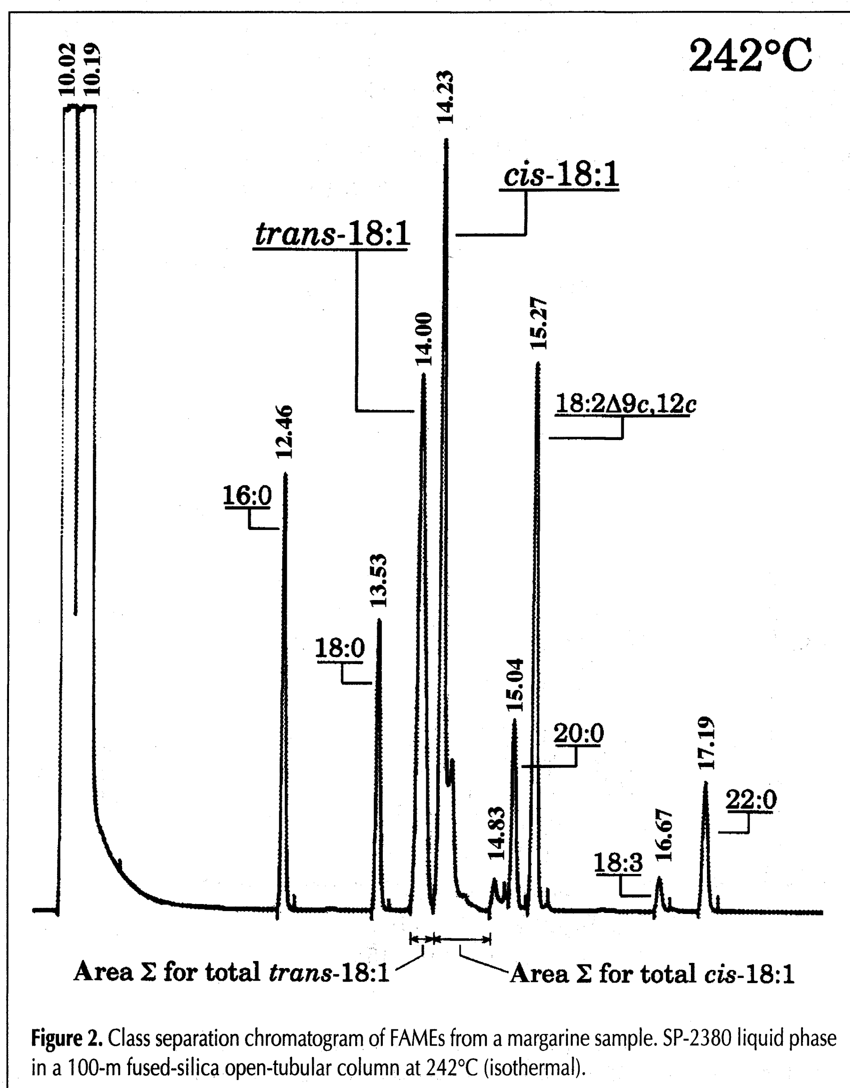

Figure 2. Class separation chromatogram of FAMEs from a margarine sample. SP-2380 liquid phase in a 100-m fused-silica open-tubular column at 242°C (isothermal).

Table I. FAMEs Identified on the Chromatogram in Figure 2

Labeled peak in Figure 2	FAME		Position and geometry of olefinic bond	ECL at 242°C*
16:0	palmitate		–	16.000
18:0	stearate		–	18.000
trans-18:1	Possible contributors:			
		18:1	5*t*	18.462
		18:1	6*t*	18.514
		18:1	7*t*	18.538
		18:1	8*t*	18.559
		18:1	(4*c*)	18.576
		18:1	9*t*	18.577
		18:1	10*t*	18.605
		18:1	11*t*	18.629
		18:1	(5*c*)	18.638
		18:1	12*t*	18.666
		18:1	14*t*	18.671
		18:1	13*t*	18.683
		18:1	15*t*	18.716
cis-18:1	Possible contributors:			
		18:1	6*c*	18.784
		18:1	7*c*	18.794
		18:1	8*c*	18.829
		18:1	9*c*	18.865
		18:1	10*c*	18.901
		18:1	11*c*	18.943
		18:1	(16*t*)	18.997
		–	(19:0)	19.000
		18:1	12*c*	19.002
		18:1	13*c*	19.068
		18:1	14*c*	19.135
		18:1	15*c*	19.182
18:2Δ9*c*,12*c*	linoleate		9*c*,12*c*	19.923
20:0	arachidate		–	20.000
18:3	linolenate		9*c*,12*c*,15*c*	21.078
22:0	behenate		–	22.000

* ECL values for 242°C on SP-2330 were determined using linear regression equations developed from the data set of Bannon et al. (13).

FAMEs contributing to the areas of these two composite peaks are listed in order of their equivalent chain length (ECL) values at 242°C.

It is evident from studying the listing in Table I that the separation is not perfect with respect to the correct classification and inclusion of all possible 18:1 isomers. There are five specific FAMEs (18:1Δ5*c*, 18:1Δ4*c*, 18:1Δ16*c*, 18:1Δ16*t*, and 19:0) in the list that might potentially confound a rapid analysis for total *trans*- and *cis*-18:1 based on the new direct, high-temperature GLC separation. Fortunately, all but one (18:1Δ4*c*) are readily quantitatable with only modest adjustments to the GLC conditions, as discussed in the next section.

For the most part, the quantitative estimation of total *trans*- and *cis*-18:1 will not be impaired by the failure of the high-temperature separation to deal seamlessly with the five troublesome FAMEs just mentioned. From a practical perspective, only 18:1Δ5*c* presents itself as a potentially noticeable source of error in work with food products whose principal source of isomers is partially hydrogenated vegetable oils. Any such concern is largely ameliorated by the numerous research reports showing that 18:1Δ5 (both *cis* and *trans*) isomers are at very low levels in nearly all such samples (3,6,11,20–27). Furthermore, when the level of 18:1Δ5*c* is low enough to be disregarded, the level of 18:1Δ4*c* is bound to be negligible, for reasons ascribable to the workings of the hydrogenation process. Based on similar reasoning and the sizable body of literature just cited, the levels of both 18:1Δ16*c* and 18:1Δ16*t* are usually too low to confound the single-step analysis. The same would be true of nonadecanoate (19:0) unless it had been incorporated into the sample as an internal standard for general fatty acid analysis.

Naturally, caution is necessary when working with ruminant fats, which contain patterns of fatty acid isomers that are much more varied in comparison to vegetable oils (partially hydrogenated). It is well-known that the five troublesome FAMEs do, in fact, exist in higher amounts in the fats of ruminate animals (7,28). Thus, for the purposes of quality control during the assay of total *cis*- and *trans*-18:1, it is imperative that there be simple and direct ways to estimate them individually. This is especially true with food compositional analyses, which often involve samples for analyses that are complex in the sense that they are mixtures (e.g., total daily diets, composited) and are accompanied with only scant information as to their component parts. The precise measurement of 18:1Δ16*t* has taken on more importance in its own right; Wolff (6) has shown that the levels of this specific isomer can be conveniently used to derive a measure of the proportion of *trans*-18:1 in one's diet that originated from the intake of ruminate fats as opposed to other sources, such as partially hydrogenated oil-based products.

Quality control: Checking for unusually high levels of 18:1Δ5*c*, 18:1Δ16*c*, 18:1Δ16*t*, and 19:0

*SP-2380 for 18:1Δ16*t

The SP-2380 column used in Figure 2 (242°C) can also be used to ascertain the levels of 18:1Δ16*t* by simply lowering the column's temperature. However, if the temperature is set too low, the 18:1Δ16*t* peak will be co-located with 18:1Δ14*c*, as it is in Figure 1 (175°C). The optimal temperature was found to be 190°C ± 2, a temperature at which 18:1Δ16*t* eluted midway between 18:1Δ13*c* and 18:1Δ14*c*. This was predicted once graphs were prepared that showed the relation between ECL and temperature (see next section and Figures 3–5).

Carbowax-20M

The levels of 18:1Δ5*c*, 18:1Δ16*c*, and 19:0 are easily checked using a Carbowax-20M column, particularly if it is operated isothermally at the two temperature settings recommended recently for simplifying and standardizing general fatty acid work (12). For example, at 183°C, all three are very well-separated from all other *trans–cis* isomeric FAMEs (R.H. Thompson. Simplifying fatty acid analyses using a standard set of gas–liquid chromatographic conditions: II. Equivalent chain length values for *cis*- and *trans*- isomers of monoethylenic C_{18} fatty acid methyl esters for Carbowax-20M liquid phase. *J. Chromatogr. Sci.*, in press.) as well as from most other types of

FAMEs (12). At 212°C, a second set of values for 18:1Δ16*c* and 19:0 was obtained. In the same chromatogram, the vanishingly small peak for 18:1Δ16*t* was sufficiently well-resolved for a reasonably good estimate to be obtained. The obtained value should agree with those obtained on the SP-2380 column at 190°C, as discussed above. Detailed information on the retention behavior of all of the 18:1 isomers on the Carbowax-20M stationary phase is the subject of another report devoted specifically to that topic (R.H. Thompson. Simplifying fatty acid analyses using a standard set of gas–liquid chromatographic conditions: II. Equivalent chain length values for *cis*- and *trans*- isomers of monoethylenic C_{18} fatty acid methyl esters for Carbowax-20M liquid phase. *J. Chromatogr. Sci.*, in press.). Other improvements may be possible if isopropyl ester derivatives (28) or butyl ester derivatives (7) are tested for their affects on these separations.

Class separation through higher temperature

The graphs shown in Figures 3–5 first revealed that temperatures substantially higher than those normally used (typically 165–185°C) should be investigated. The lines in the figures are the linear least-square lines for the relation between ECL and column temperature for each of the FAMEs with available data. The original ECL information used to prepare these graphs came from Bannon et al. (13), who published extensive ECL information at three different temperatures (180, 200, and 220°C) rather than only one, as is commonly done. That permitted ECL information to be processed according to regression procedures and plotting procedures previously described in a report on ECL shifts using another phase, Carbowax-20M (12). Consistent with that earlier study on Carbowax-20M (12), the dependency of ECL on column temperature was precisely linear (the square of the correlation coefficient [r^2] was greater than 0.99), highly reproducible, and characteristic for a given FAME.

Figures 3 and 4 show this relationship (dependency of ECL on temperature) for *cis*-18:1 and *trans*-18:1 FAMEs, respectively. Shading has been applied to these two figures and subsequent ones to indicate which FAMEs make up the peaks for total *cis*-18:1 and *trans*-18:1 (i.e., the *cis*-18:1 peak and *trans*-18:1 peak in the class separation chromatogram displayed in Figure 2). In the case of the 12 *cis*-18:1 FAMEs (Figure 3), 10 of their number are shaded (18:1Δ6*c*–18:1Δ15*c*), indicating that they contribute to the (total) *cis*-18:1 peak area (i.e., the *cis*-18:1 peak in Figure 2). Similarly, for the 12 FAMEs in the *trans*-18:1 family plotted in Figure 4, 11 (18:1Δ5*t*–18:1Δ15*t*) were contributors to the (total) *trans*-18:1 peak area (i.e., the *trans*-18:1 peak in Figure 2).

In Figure 5, the lines in Figures 3 and 4 have been combined to illustrate the basis for the class separation that occurs at a high temperature (242°C). This pleasing result was derived from the differences in the slopes of the lines: the shifts in ECL were more pronounced for the *cis*-18:1 family of isomers than for their *trans* counterparts (compare slopes of lines in Figures 3 and 4). Consequently, the two shaded areas diverge on the ECL scale as one moves further to the right in Figure 5. Even-

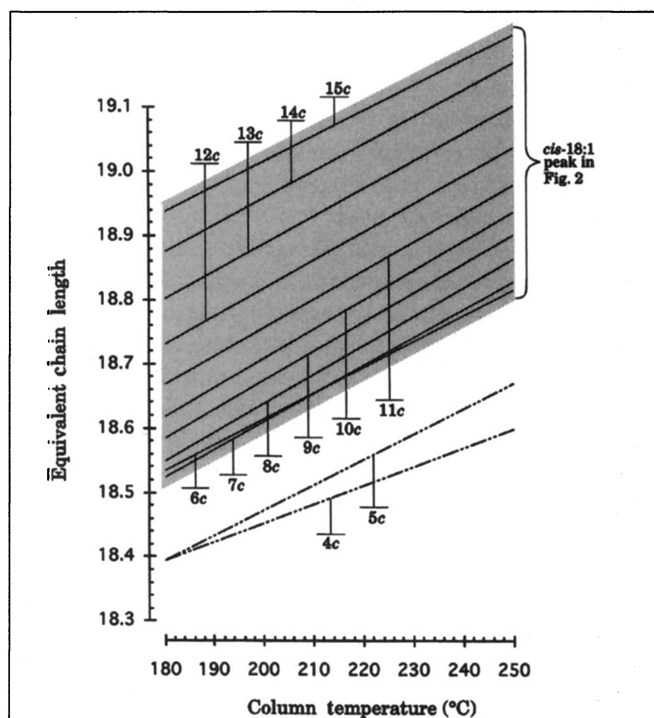

Figure 3. Dependence of ECL on column temperature for 12 different *cis*-18:1 FAMEs. Positional isomers 18:1Δ6*c*–18:1Δ15*c* (shaded region) make up the composite peak identified as the *cis*-18:1 peak in Figure 2. The remaining two, 18:1Δ4*c* and 18:1Δ5*c* (dashed lines), are co-located with the *trans*-18:1 peak. Not appearing is 18:1Δ16*c*; it appeared later in the run (see Figure 6).

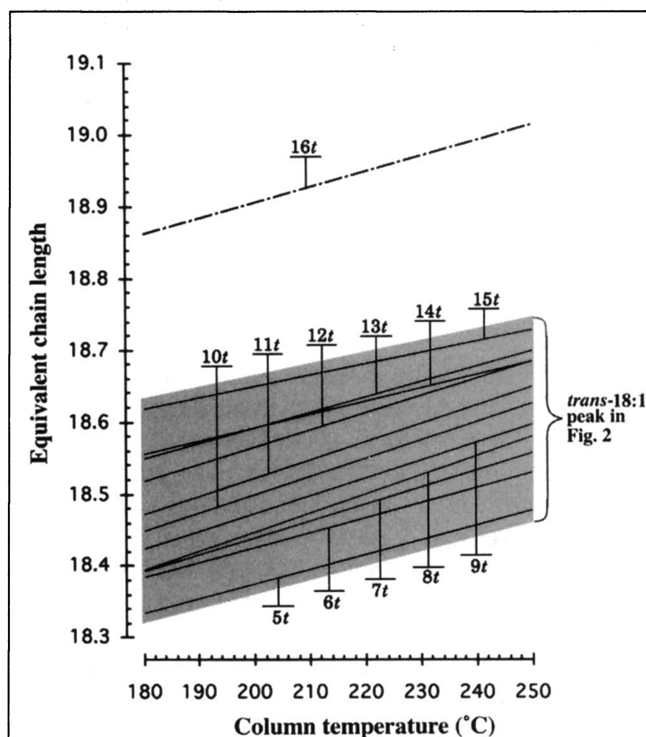

Figure 4. Dependence of ECL on column temperature for 12 different *trans*-18:1 isomers, 11 of which were included in the *trans*-18:1 peak during the high-temperature class separation (see Figure 2). The single outlier, 18:1Δ16*c* (dashed line), is co-located with the *cis*-18:1 peak.

tually, at approximately 230°C, a small triangular region (far right, not shaded) began to develop and then eventually widen to the point that a baseline separation occurred in the chromatogram at approximately 242°C (Figure 2).

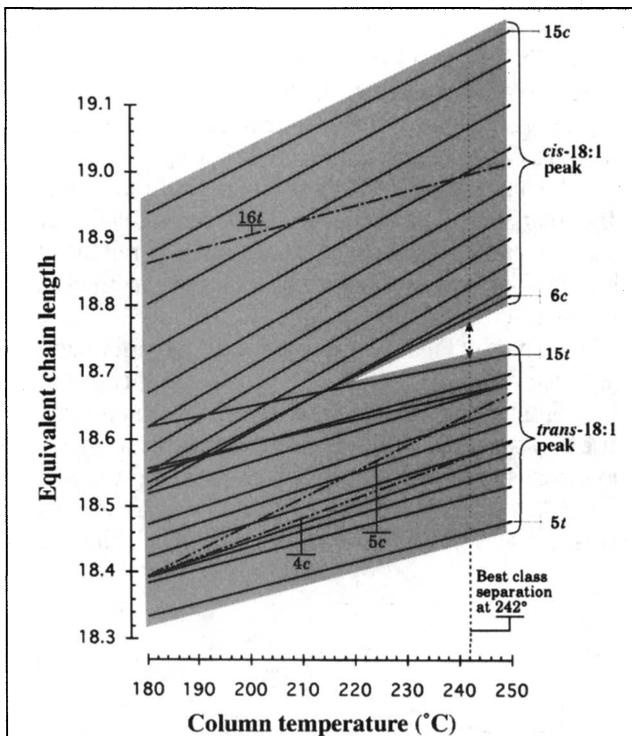

Figure 5. Dependence of ECL on column temperature for all 24 of the 18:1 isomers displayed together, showing the basis for the 18:1 class separation. The best separation between the *trans*-18:1 peak and the *cis*-18:1 peak occurred at 242°C (see short vertical line with arrows).

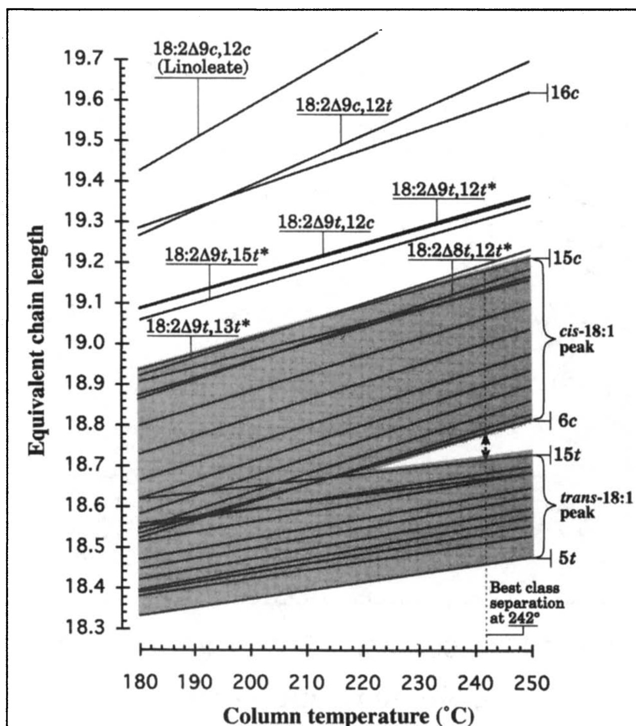

Figure 6. Dependence of ECL on column temperature for 18:1 isomers and seven different 18:2 isomers. 18:2*t,t* FAMEs are marked with asterisks.

When the column temperature was pushed even higher in the hope of further maximizing the separation between the two classes, a disappointing result was obtained. Above approximately 244°C, the benefits began to be nullified by the diminution in the column's partition ratio (or capacity ratio [*k*]), a measure of the time that the molecules of a given species spend in the liquid phase relative to their time in the gas phase (29). Thus, at excessively high temperatures, there simply was insufficient opportunity for the FAME molecules to interact with the stationary phase for the class separation to occur. At temperatures above approximately 252°C, the resolution between the *trans*-18:1 and *cis*-18:1 peaks (Figure 2) was marginal at best, and it deteriorated further with temperatures higher than that. The optimum temperature range was found to be 242°C ± 2, which represents a compromise between the two aforementioned temperature-dependent effects: better class separation with increasing temperatures (due to the alteration of liquid-phase polarity) versus decreasing *k* values with increasing temperatures.

18:1Δ5c, 18:1Δ4c, 18:1Δ16c, 18:1Δ16t, and 19:0

Figures 3–6 are also useful for visualizing how these (trace-level) FAMEs could potentially cause problems during "direct" GLC analysis of total *trans*-18:1 and *cis*-18:1 and why the previously described quality control measures should be made a part of routine analyses, particularly for work with complex mixtures. For example, in Figure 3, neither 18:1Δ4*c* nor 18:1Δ5*c* (both displayed with dashed lines) fall in the shaded region that identifies the contributors to the *cis*-18:1 peak in the class separation (Figure 2). Instead, both of these *cis* acids are co-located with the *trans*-18:1 peak (cf. Figures 3 and 5), and it is obvious that no simple temperature adjustment can remedy this deficiency. Notice also that 18:1Δ16*c* is absent from the shaded area of Figure 3, and consequently, it was not included in the figure obtained for (total) *cis*-18:1; its point of elution was substantially later in the chromatographic run (see the discussion of Figure 6 in the next section). In Figure 4, 18:1Δ16*t* (dashed line) is not a part of the shaded area, so its contribution to the *trans*-18:1 peak was not properly reported. Again, no simple temperature alteration will resolve this problem, as 18:1Δ16*t* will be co-located with the *cis*-18:1 peak (Figures 2 and 5) regardless of the temperature setting of this particular column. The ECL-versus-temperature relationship for 19:0 is simply represented by a horizontal line (slope = 0) crossing the ECL axis at 19.0. At that position on Figure 3, it would (if it is present) contribute to the *cis*-18:1 peak.

18:2 isomers in the "direct" GLC chromatogram

One question that is sure to be asked is where the 18:2 isomers appear on the class separation chromatogram, and to what extent they pose a problem in the "direct" GLC determination of (total) *cis*-18:1 and *trans*-18:1. Figure 6 was designed to answer this question with the presentation of linear least-square lines for seven additional FAMEs, all 18:2 isomers, taken from the work of Bannon et al. (13). Their addition to the display required that the *y*-axis (ECL) be expanded out to 19.7 rather than stopping at 19.1, as in Figures 3–5. Figure 6 clearly

shows that linoleate (18:2Δ9*c*,12*c*) was well-removed from either of the composite 18:1 peaks; this is evident in the actual chromatogram shown in Figure 2 as well. All of the 18:2 positional isomers named in a comprehensive report on *trans* fatty acids in human milk (30) are represented in this group of seven 18:2 FAMEs, those being Δ9,12; Δ8,12; Δ9,13; and Δ9,15. Although specimens were not available to record elutions for all their possible geometric isomers (four for each), important information can be deduced from the pattern of elutions for the set supplied by Bannon et al. (13). For each set of double bonds (positions), the shortest retention time (smallest ECL) was with *trans,trans*; the other three geometic isomers eluted later than *trans,trans*. Bannon et al. (13) provided ECLs for the *trans,trans* component in each of the positional categories listed above. By being the earliest to elute in each positional family, these four 18:2*t,t* FAMEs (marked with asterisks in Figure 6) conveniently delineate the worse case of overlap or interference with the *cis*-18:1 peak in the class separation chromatogram (Figure 2). Two of them, 18:2Δ9*t*,13*t* and 18:2Δ8*t*,12*t*, appeared closest to the the *cis*-18:1 peak and therefore posed the maximal threat to the analysis. All the others were well-removed from the composite peaks used to obtain (total) *cis*-18:1 and *trans*-18:1.

Factors influencing column choice

Three different cyanopropyl liquid phases (SP-2330, SP-2380, and SP-2560) with differing degrees of cyanopropyl substitution were used for this study of FAME elutions. According to Bannon et al. (13), their ECL information was collected using SP-2330 (nonbonded), the least polar of the three, with just 80% substitution (cyanopropyl for phenyl). It is their elution data on SP-2330 that is represented by the linear least-square lines in Figures 3–6. The margarine FAME separation shown in Figure 1 was made with SP-2560 (nonbonded), which is described by its manufacturer as being specifically designed to provide separation of geometric-positional (*cis* and *trans*) isomers of FAMEs. Specific information as to its cyanopropyl content was not provided by the manufacturer, but one can assume it to be at least 90%, based on a comparison with the other two phases, as discussed below in connection with Figure 7.

The custom column that was requested from the manufacturer for the express purpose of pursuing a "direct" class (*cis*- and *trans*-18:1) separation was made with SP-2380. The distinguishing feature of this particular phase that drew this author's attention was its description as "stabilized", presumably meaning it is crosslinked or crossbonded in situ. One would then expect it to possess greater stability at elevated temperatures compared with the three other more traditional, nonbonded, cyanosilicone-type phases offered by the same manufacturer (SP-2330, SP-2340, and SP-2560). Several manufacturers were approached about the custom column, but only one showed any interest or capability in preparing it according to the specifications requested.

The specifications for the custom column called for it to be somewhat out of the ordinary in two respects: inner diameter and film thickness. Both were adjusted to give a larger *k* value to begin with to counteract the loss in capacity ratio accompanying its sustained operation at temperatures expected to be as much as 70–90°C above those traditionally used for FAMEs on such columns. A film thickness of 0.30 μm was specified, rather than 0.20 μm, which is more typically used for *cis* and *trans* isomer applications. The internal diameter of the fused-silica capillary was specified to be 0.20 mm for the custom column, whereas 0.25 mm is typically used.

The purpose of Figure 7 was to contrast the performances of the three columns mentioned above. Only two FAMEs, 18:1Δ15*t* and 18:1Δ6*c*, were plotted for this comparison because they conveniently define the innermost boundaries of the two composite peaks (the *trans*-18:1 peak and the *cis*-18:1 peak, Figures 5 and 6) that are to be used to quantitate (total) *trans*- and *cis*-18:1 acids. Fundamentally, the picture was the

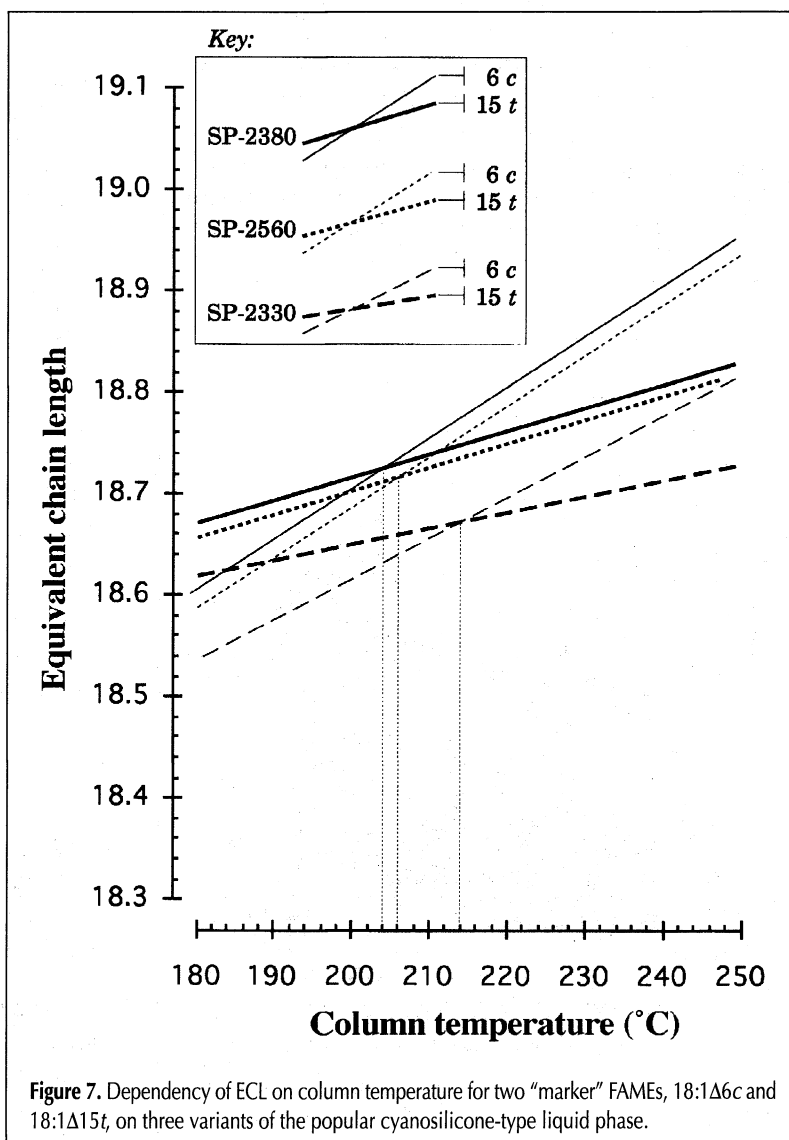

Figure 7. Dependency of ECL on column temperature for two "marker" FAMEs, 18:1Δ6*c* and 18:1Δ15*t*, on three variants of the popular cyanosilicone-type liquid phase.

same for all three columns (Figure 7), except that some differences could be seen in the "polarity" of the three phases. For example, there was a difference in the temperature at which the lines for the two marker FAMEs crossed each other. The more polar the column, the lower the temperature at which the crossover occurred. On this basis, the SP-2380 phase (custom column) with a crossover point at approximately 204°C was only slightly more polar than the SP-2560 phase, which had its crossover at approximately 206°C. The SP-2330 phase, on the other hand, with its crossover at approximately 214°C, is substantially less polar than either of the other two, which is to be expected, given its 80% cyanopropyl substitution, as compared with 90% for SP-2380. Part of the observed differences between SP-2330 (Australian laboratory of Bannon et al.) and SP-2380 and SP-2560 (author's laboratory) may well be due to differences in temperature calibration of the chromatographs.

Conclusion

The new GLC class separation (Figure 2) correctly classifies 20 of the 24 different *cis*- and *trans*-isomeric C_{18} fatty acids. The four problematic ones can be disregarded inasmuch as they are very minor contributors to their respective totals, particularly in working with partially hydrogenated vegetable oils. FAMEs of the 18:2 and 18:3 variety (including all the possible positional and conformational isomers) did not interfere; they all eluted much later in the chromatographic run. Further assurances as to the insignificance of the levels of Δ5 *cis*, Δ4 *cis*, Δ16 *cis*, and Δ16 *trans* as well as nonadecanoate can be gained by measuring them directly using recently published guidelines on the Carbowax-20M phases (12,31, R.H. Thompson. Simplifying fatty acid analyses using a standard set of gas–liquid chromatographic conditions: II. Equivalent chain length values for *cis*- and *trans*- isomers of monoethylenic C_{18} fatty acid methyl esters for Carbowax-20M liquid phase. *J. Chromatogr. Sci.*, in press.).

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