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

Raymond H. Thompson

Beltsville Human Nutrition Research Center, Food Composition Laboratory, U.S. Department of Agriculture, Building 161, BARC-East, Beltsville, MD 20705

Abstract

The Carbowax-20M liquid phase is becoming the standard in gas chromatography for general fatty acid analyses because it simplifies the work and reduces the chance for errors in identification of fatty acids. Isothermal analysis using the Carbowax-20M column, as opposed to temperature-programming, provides further simplification and ruggedness to the analysis. An extensive compilation of characteristic separation factors for 59 different fatty acid methyl esters has appeared, but it included gas–liquid chromatography elution information on only four of the approximately 20–24 isomeric octadecenoates that occur in foods containing ruminate fats and partially-hydrogenated vegetable oils. This contribution serves to fill that void by providing the equivalent chain length elution factors for 25 additional *trans*- and *cis*-monoethylenic fatty acid methyl esters.

Introduction

In the identification and quantitation of fatty acids using capillary gas–liquid chromatography (GLC), standardization on a PEG-type (poly[ethylene glycol]) liquid-phase generally results in more understandable chromatograms and, hence, fewer errors in the assignment of peak identities. Ackman has made the case using numerous examples and has recommended that such columns be the standard for general fatty acid work (1,2). In contrast to the more polar cyanosilicone phases, the elution order of fatty acid methyl esters (FAMEs) on PEG is more regular with respect to the influence of carbon chain length, and therefore difficulties in interpretation resulting from chain length overlap are largely eliminated (3). PEG's polarity is sufficient to split apart the myriad FAMEs in complex samples, which the phenylsilicone phases cannot do, but it is not so great as to cause the overlaps seen with cyanosilicones. In their modern incarnations, PEG phases are coated in flexible fused silica and "bonded" or "crosslinked" in situ for stability, after which they are basically equivalent to the traditional Carbowax-20M in selectivity toward FAMEs.

Using PEG phases, further simplifications are possible when analyses are carried out under constant column oven temperatures (i.e., no temperature-programming except perhaps for only the simplest of FAME mixtures) (4). With GLC runs made at a constant temperature, the equivalent chain length (ECL) system can be used to represent the retention behavior very precisely for each FAME. Also, for each (straight-chain) FAME, the relation between ECL and temperature is precisely linear and unique (slope and y-intercept), allowing one to compare not only absolute ECLs, but also the changes in ECLs (ΔECL) after repeated chromatography at other (constant) temperature settings.

The use of ECLs and ΔECLs in the interpretation of complicated chromatograms has proven to be a valuable technique, particularly in the analysis of multicomponent foods and mixtures such as (composited) total daily diets. Part I of this work (4) stressed the disadvantages accompanying the overuse of temperature programming and listed several benefits to the routine use of two specific isothermal temperatures, 183 and 212°C, for reducing confusion and errors in the identification of individual FAMEs in complicated mixtures and for generally simplifying and standardizing the process of fatty acid analysis. It not only furnished ECLs for those specific preferred temperatures, but also contained the slope and intercept constants for 59 different FAMEs on a PEG phase so that their elutions could be easily calculated for any other temperature. The information presented herein is intended to supplement that ECL resource to include reference values for 26 additional compounds, specifically the *cis*- and *trans*-monoethylenic C_{18} isomers (*cis*- and *trans*-18:1).

A second objective was to illustrate how the expanded refer-

Table I. Regression Equation Constants* and ECL Values for Selected Column Temperatures†

Fatty acid	ECL values Preferred temperatures			Regression analysis: ECL versus temperature				ECL values (Compare with published values**)			
				Saturated	Coefficients§ Slope	Intercept					
	183°C	212°C	247°C	FAME‡	($m \times 10^3$)	(b)	R^2	175°C	180°C	195°C	200°C
cis-4	18.209	18.237	18.271	16,20	0.975	18.030	1.000	18.201	18.206	18.220	18.225
cis-5	18.121	18.165	18.219	16,20	1.525	17.842	1.000	18.109	18.117	18.140	18.147
cis-5	**18.124**	**18.167**	**18.220**	**16,18,20**	**1.489**	**17.852**	**0.993**	**18.112**	**18.120**	**18.142**	**18.150**
cis-6	18.194	18.234	18.281	16,20	1.350	17.947	1.000	18.184	18.190	18.211	18.217
cis-6	**18.195**	**18.236**	**18.284**	**16,18,20**	**1.394**	**17.940**	**0.996**	**18.184**	**18.191**	**18.212**	**18.219**
cis-7	18.167	18.210	18.263	16,20	1.500	17.892	1.000	18.155	18.162	18.185	18.192
cis-8	18.176	18.221	18.275	16,20	1.550	17.892	1.000	18.163	18.171	18.194	18.202
cis-9	18.198	18.241	18.294	16,20	1.500	17.923	1.000	18.186	18.193	18.216	18.223
cis-10	18.225	18.268	18.320	16,20	1.475	17.956	0.999	18.214	18.221	18.243	18.251
cis-11	18.268	18.308	18.357	16,20	1.400	18.011	1.000	18.256	18.263	18.284	18.291
cis-11	**18.261**	**18.311**	**18.372**	**16,18,20**	**1.739**	**17.942**	**0.981**	**18.247**	**18.255**	**18.281**	**18.290**
cis-12	18.328	18.366	18.412	16,20	1.325	18.085	1.000	18.317	18.324	18.344	18.350
cis-12	**18.325**	**18.368**	**18.419**	**16,18,20**	**1.470**	**18.056**	**0.994**	**18.313**	**18.321**	**18.343**	**18.350**
cis-13	18.396	18.432	18.475	16,20	1.225	18.172	1.000	18.387	18.393	18.411	18.417
cis-13	**18.395**	**18.433**	**18.479**	**16,18,20**	**1.315**	**18.155**	**0.996**	**18.385**	**18.391**	**18.411**	**18.418**
cis-14	18.478	18.510	18.550	16,20	1.125	18.272	1.000	18.469	18.474	18.491	18.497
cis-15	18.563	18.591	18.625	16,20	0.975	18.384	1.000	18.555	18.560	18.574	18.579
cis-16	18.876	18.910	18.950	16,20	1.150	18.666	1.000	18.867	18.873	18.890	18.896
trans-5	18.219	18.241	18.267	16,20	0.750	18.082	0.994	18.213	18.217	18.228	18.232
trans-5	**18.218**	**18.243**	**18.274**	**16,18,20**	**0.873**	**18.058**	**0.949**	**18.211**	**18.216**	**18.229**	**18.233**
trans-6	18.225	18.244	18.267	16,20	0.650	18.106	0.998	18.220	18.223	18.233	18.236
trans-6	**18.225**	**18.245**	**18.268**	**16,18,20**	**0.664**	**18.104**	**0.994**	**18.220**	**18.223**	**18.233**	**18.237**
trans-7	18.207	18.231	18.260	16,20	0.825	18.056	0.997	18.201	18.205	18.217	18.221
trans-7	**18.216**	**18.241**	**18.270**	**16,18,20**	**0.849**	**18.061**	**0.988**	**18.209**	**18.214**	**18.226**	**18.231**
trans-8	18.212	18.236	18.265	16,20	0.825	18.062	0.997	18.206	18.210	18.222	18.227
trans-8	**18.214**	**18.242**	**18.275**	**16,18,20**	**0.961**	**18.038**	**0.962**	**18.206**	**18.211**	**18.225**	**18.230**
trans-9	18.214	18.239	18.270	16,20	0.875	18.054	0.993	18.207	18.211	18.224	18.229
trans-9	**18.223**	**18.249**	**18.281**	**16,18,20**	**0.900**	**18.058**	**0.991**	**18.216**	**18.220**	**18.234**	**18.238**
trans-10	18.238	18.262	18.290	16,20	0.800	18.092	1.000	18.232	18.236	18.248	18.252
trans-10	**18.242**	**18.266**	**18.295**	**16,18,20**	**0.831**	**18.090**	**0.979**	**18.235**	**18.239**	**18.252**	**18.256**
trans-11	18.262	18.284	18.310	16,20	0.750	18.125	0.994	18.256	18.260	18.271	18.275
trans-11	**18.264**	**18.285**	**18.311**	**16,18,20**	**0.724**	**18.132**	**0.969**	**18.259**	**18.262**	**18.273**	**18.277**
trans-12	18.296	18.314	18.336	16,20	0.625	18.181	0.999	18.291	18.294	18.303	18.306
trans-12	**18.299**	**18.319**	**18.344**	**16,18,20**	**0.691**	**18.173**	**0.925**	**18.294**	**18.297**	**18.308**	**18.311**
trans-13	18.338	18.353	18.370	16,20	0.500	18.247	0.987	18.334	18.337	18.344	18.347
trans-13	**18.343**	**18.360**	**18.381**	**16,18,20**	**0.592**	**18.234**	**0.977**	**18.338**	**18.341**	**18.350**	**18.353**
trans-14	18.352	18.362	18.374	16,20	0.350	18.288	0.974	18.349	18.351	18.356	18.358
trans-15	18.433	18.434	18.436	16,20	0.050	18.424	0.429	18.432	18.433	18.433	18.434
trans-15	**18.444**	**18.444**	**18.445**	**16,18,20**	**0.023**	**18.439**	**0.121**	**18.443**	**18.444**	**18.444**	**18.444**
trans-16	18.659	18.663	18.669	16,20	0.150	18.632	0.871	18.658	18.659	18.661	18.662
delta-17	18.550	18.568	18.590	16,20	0.625	18.436	0.999	18.545	18.548	18.558	18.561
8*t*, 12*t*	18.487	18.524	18.569	16,20	1.275	18.254	1.000	18.477	18.483	18.502	18.509
9*c*, 12*c*	18.650	18.715	18.793	16,20	2.225	18.243	1.000	18.633	18.644	18.677	18.688
9c, 12c	**18.645**	**18.729**	**18.831**	**16,18,20**	**2.911**	**18.112**	**1.000**	**18.622**	**18.636**	**18.680**	**18.694**
9*c*, 12*t*	18.704	18.749	18.805	16,20	1.575	18.416	0.999	18.691	18.699	18.723	18.731
9*t*, 12*c*	18.689	18.710	18.735	16,20	0.725	18.556	1.000	18.683	18.687	18.698	18.701
9c, 12t	**18.776**	**18.824**	**18.881**	**16,18,20**	**1.638**	**18.476**	**0.994**	**18.763**	**18.771**	**18.796**	**18.804**
9*t*, 12*t*	18.688	18.710	18.736	16,20	0.750	18.551	0.994	18.682	18.686	18.697	18.701
9*t*, 13*t*	18.535	18.568	18.608	16,20	1.150	18.324	0.999	18.526	18.531	18.549	18.554
9*t*, 15*t*	18.662	18.683	18.708	16,20	0.725	18.529	1.000	18.656	18.660	18.671	18.674
10*t*, 12*t*	20.115	20.158	20.209	16,20	1.475	19.845	1.000	20.103	20.111	20.133	20.140
18:3	19.278	19.361	19.462	16,20	2.875	18.751	1.000	19.255	19.269	19.312	19.326

* Slope (m) and y-intercept (b).

† Linear least-square information developed from the ECL values of Bannon et al. (6) are shown in plain type; those in bold are from this study's chromatograph.

‡ Within each row, ECL calculations were based on the retentions of the three saturated FAMEs identified in this column using the notation n_1, n_2, n_3, where n_1, n_2, and n_3 refer to the number of carbons in the fatty acid backbone. For example, "14,16,18," would indicate that 14:0, 16:0, and 18:0 were the FAMEs used to determine ECL.

§ Values for m and b were used for prediction of the ECL for FAMEs at specific temperatures.

** These temperatures are listed to make it convenient to compare ECL values with those of Christie (7) (cf. 175°C), Ackman (2) (cf. 180°C), Kramer et al. (8) (cf. 195°C), and Bannon et al. (6) (cf. 200°C).

ence of ECLs may be used in the solution of specialized separation problems, using as an example some successes with the use of PEG capillary columns for validation and quality control (QC) monitoring during the development of an 18:1 *cis-/trans*-class separation and quantitation (direct GLC) method (5). In that connection, specific examples were given showing the separations of 18:1Δ5 *cis*, 18:1Δ16 *cis*, 18:1Δ16 *trans*, and 19:0 with the same column at the preferred temperatures (183 and 212°C) mentioned above and stressed in Part I in connection with general fatty acid work.

Experimental

Materials

Fatty acid methyl 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 added methyl eicosanoate and methyl docosanoate was purchased from Supelco (Bellefonte, PA).

GLC

The chromatograph was a Hewlett-Packard model 5840 with a flame-ionization detector and a split–splitless capillary inlet. Injector and detector temperatures were 250 and 275°C, respectively. Helium and hydrogen carrier gases (ultrapure carrier, 99.999%) from Air Products and Chemicals (Allentown, PA) were further purified with traps of charcoal, molecular sieve, and oxygen scrubber as previously described (4). Carrier linear velocity was set to approximately 20 cm/s for helium and approximately 38 cm/s for hydrogen at the column operating temperature. Sample injections were made with a 10-µL syringe using a split ratio of approximately 100:1. The injection liners were deactivated inlet sleeves (cup-splitter type [Restek, Bellefonte, PA]) packed with approximately 0.5 cm of GLC packing (1% JXR on Gas Chrom Q [Restek]) and held in place by small plugs of deactivated fused-silica wool (Restek) above and below (4).

The GLC column was STABILWAX (crossbonded Carbowax-20M) on a fused-silica capillary (60 m × 0.25-mm i.d.) with a phase thickness of 0.25 mm (Restek). The retention times were those reported by the chromatograph and represent the time from the injection of the sample to the apex of the peaks. The conversion of retention time data to ECL values was done on a Macintosh 8100 computer using Microsoft Excel spreadsheet software, as previously described (4). The saturated FAMEs used for ECL determination were 16:0, 18:0, and 20:0; the correction for holdup time was made using a theoretical (calculated) retention time for an unretained solvent (4).

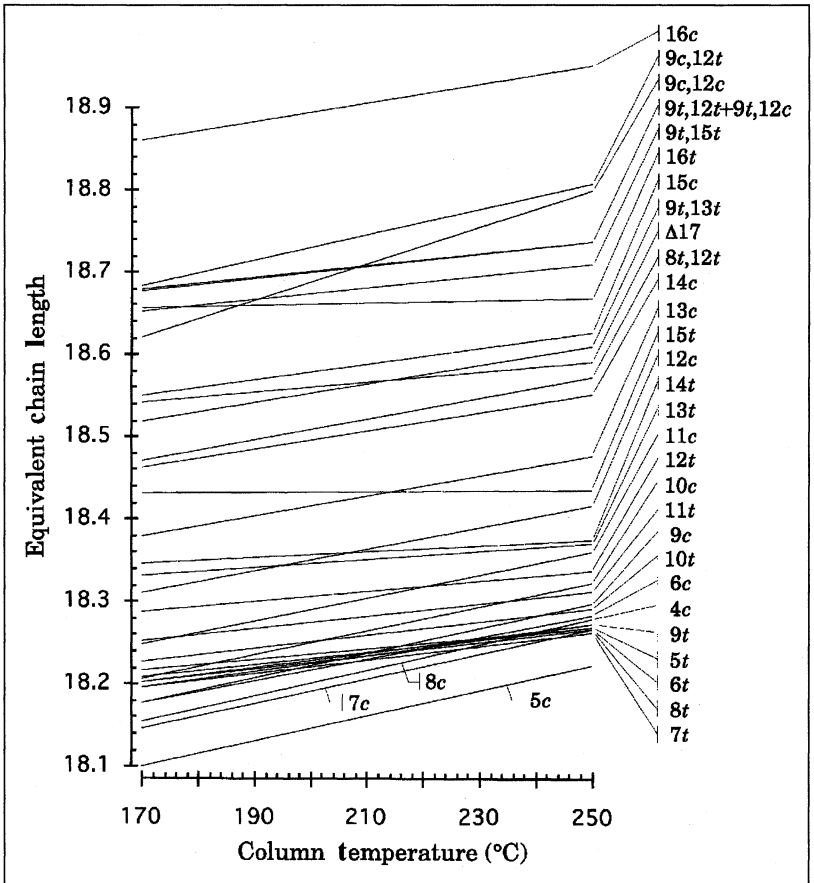

Figure 1. Graph showing the dependence of ECL on column temperature for C_{18} isomers, prepared using regression analyses applied to a database of ECL values collected at various temperatures as previously described (4).

Results and Discussion

An ECL supplement to facilitate work with *cis-* and *trans-* fatty acids

The ECL information in Table I was derived from three sources: (*a*) Available standards were repeatedly analyzed by GC at temperature intervals of approximately 2–5°C (over the range 170–240°C), ECLs were calculated, and results were reduced to linear least-square lines representing the ECL–temperature relation using previously described procedures (4). (*b*) Margarine extracts were repeatedly analyzed in the same way to expand the variety of fatty acids that could be studied. (*c*) The meticulous ECL data published by Bannon et al. (6) were subjected to evaluation and regression analysis to see if the data from the two separate laboratories could legitimately be combined into the single table for convenient reference. The graphical display in Figure 1 showing and emphasizing the dependency of ECL on column temperature for 18:1 isomers augments similar information on 59 other FAMEs that appeared in Part I (4).

The ECL values derived from chromatography in the author's laboratory were based on three saturated (benchmark) FAMEs (16:0, 18:0, and 20:0), whereas those of Bannon et al. (6) were based on two (16:0 and 20:0). In both locations, the customary corrections for column dead time

were made using mathematically determined dead time (4,6).

The very slight differences observed in ECL values from the two different locations may be explained as easily by the existence of some discrepancies in oven temperature as by any real differences in column polarity between the two PEG-type phases (STABILWAX in Beltsville, MD and SUPELCOWAX-10 in Australia). In any case, the displays of the information from both sources showed remarkably good agreement; hence, in preparing the ECL reference (Table I), the linear equations developed from chromatography in this laboratory (data shown in bold) have been combined with those derived from the data of Bannon et al. (6). (The quantity of analyte in the column has an influence on its ECL [6], which the analyst should be aware of, but that fact has not diminished the usefulness of the ECL factors [Table I] in their practical application). To be of maximal use in selecting temperatures for specific separation problems or for interpretation of chromatograms, it is important that the information contained in Table I be displayed graphically, as in Figure 1, for example (discussed immediately below).

Visual displays of ECL versus temperature

Three very useful approaches to the GLC of FAMEs have come to light through the preparation of plots similar to the one in Figure 1. (*a*) Chromatograms for complicated mixtures are more easily interpreted with fewer mistakes if general fatty acid analyses are made on PEG phases using two isothermal runs at two specific temperatures, 183 and 212°C (4). (*b*) A total of 21 different C_{18}-monoenoic isomers (10 *cis*- positional isomers plus 11 *trans*- positional isomers) are class-separable at 242°C on cyanosilicone phases, yielding two composite chromatographic peaks that are easily measured and converted to figures representing total *trans*-18:1 and total *cis*-18:1 fatty acids (5). (*c*) The same conditions under *a* (PEG capillary column at 183 and 212°C) are uniquely capable for assessing the efficacy of the new *cis-/trans*-class separation mentioned under *b*; the balance of the following discussion elaborates on this aspect.

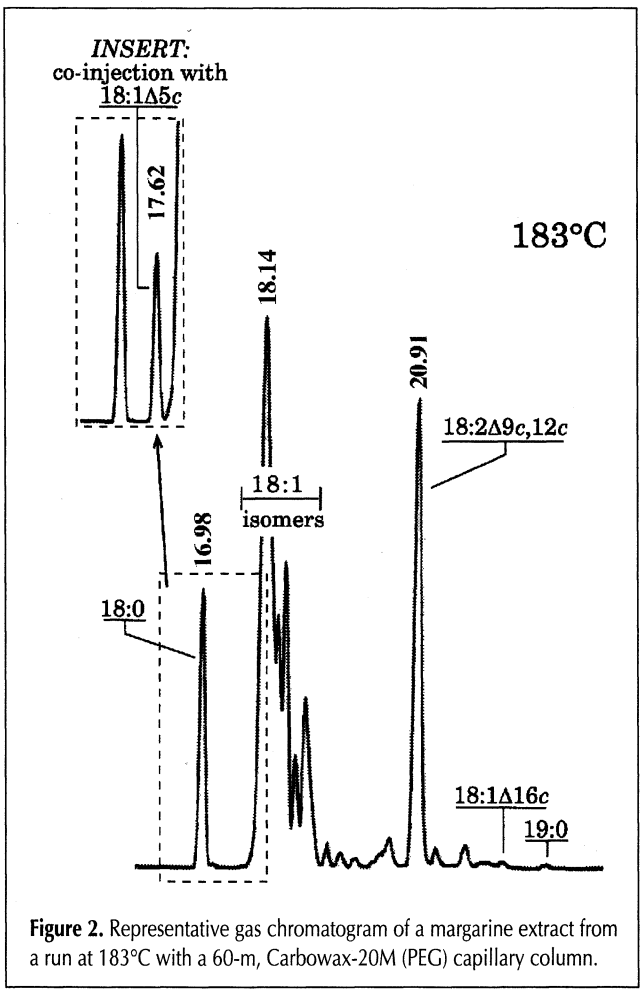

Figure 2. Representative gas chromatogram of a margarine extract from a run at 183°C with a 60-m, Carbowax-20M (PEG) capillary column.

Separations on PEG as a QC adjunct to the *cis-/trans*-18:1 class separation

Background

Published GLC experiments suggest quite strongly that a new, very rapid method for quantitation of total *trans*-18:1 (and total *cis*-18:1) may be within sight (5). Now something as simple as raising the column's temperature by some 60+°C — a marked change in the GLC realm — may provide the basis for the direct and unambiguous determination of total *trans*- and total *cis*-18:1 using GLC alone. The method currently being developed involves a single GLC analysis under 25 min, during which all of the predominant *trans*- and *cis*-C_{18}-monoethylenic isomers are separated by class into two easily measurable (composite) peaks. Of all of the 18:1 isomers known to exist in the food supply at levels above normal detection limits (no more than 26), there are only four that are not properly dealt with by the new method, and these are at such low levels that they are of little concern if current literature is accurate. Nevertheless, full documentation regarding the influence of these four acids on the efficacy of the new method was considered essential for

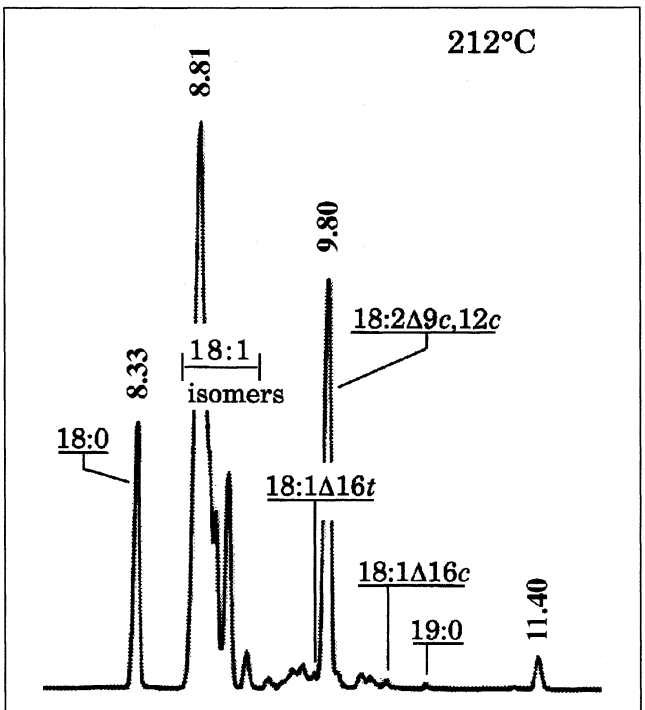

Figure 3. Representative gas chromatogram of a margarine extract from a run at 212°C with a 60-m, Carbowax-20M (PEG) capillary column.

its validation. After plots such as Figure 1 suggested that a PEG-type phase may give adequate separations, it was confirmed that this liquid phase will resolve three of the four troublesome FAMEs (18:1Δ5 *cis*, 18:1Δ16 *cis*, and 18:1Δ16 *trans*, but not 18:1Δ4 *cis*) enough to allow them to be estimated.

Measurement of 18:1Δ5 cis *on PEG*

This FAME is easily quantitated at both of the preferred temperatures established for general fatty acid work using PEG capillary columns, 183 (Figure 2) and 212°C (Figure 3). This result was expected because of the remote position of its ECL–temperature line in Figure 1.

Measurement of 18:1Δ16 cis *on PEG*

Referring again to Figure 1, the estimation of the level of this very minor contributor should be relatively easy to obtain at both of the preferred temperatures, given the remote position of its ECL–temperature line. This was the case, as can be seen in the chromatograms of Figures 2 and 3.

Rough approximation of the level of 18:1Δ16 trans *on PEG*

The column temperature must be precisely set to 212°C ± 2 in order to see a peak for this minor FAME (see Figure 3). At lower temperatures, this very small peak merges with the large peak for linoleate (18:2Δ9*c*, 12*c*), as one would expect from the way the lines converge in Figure 1. Higher temperatures cause it to merge with 18:1Δ15 *cis*.

Position of 19:0

FAME 19:0 has been marked in the two chromatograms (Figures 2 and 3) to indicate how easily its level can be estimated. This is important because 19:0 is the only other FAME identified so far that may complicate the new *cis-/trans*-18:1 GLC method by coeluting with the composite *cis*-18:1 peak (5).

Conclusion

The original "double-run" GLC strategy (4) for handling complicated FAME mixtures has been expanded upon to accommodate *cis*- and *trans*-18:1 isomer measurements. The first and second GLC steps remain the same: run 1, 183°C on a 60-m PEG capillary column; run 2 (if the complexity of the sample dictates a need for it), 212°C on the same column. The presence of *cis-/trans*- isomers will be evident because of their distinctive scatter pattern seen on both PEG runs (see areas marked as 18:1 isomers in Figures 2 and 3). If present, these are quantitated as total *cis*-18:1 and total *trans*-18:1 using a second capillary column containing a cyanosilicone liquid phase operated at 242°C (5), after verifying that the few aforementioned interfering FAMEs are at their usual ultra-trace levels using the first two chromatograms for the analyses at 183 and 212°C on the PEG column.

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