Analysis of *cis*- and *trans*-Fatty Acid Isomers in Hydrogenated and Refined Vegetable Oils by Capillary Gas–Liquid Chromatography

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ABSTRACT: For quantitation of *cis*- and *trans*-fatty acid isomers, infrared (IR) spectroscopy, gas–liquid chromatography (GLC) on highly polar stationary phases or the combination (GLC–IR) may be used. IR offers the advantage of simplicity and speed, but the lower determination limit of 5% and the lack of detailed information limit its use. Detailed fatty acid information, required for, e.g., food-labeling purposes, can only be obtained with GLC methods. Most of the GLC methods are optimized for partially hydrogenated samples. AOCS Official Method Ce 1c-89 prescribes a single, highly polar stationary phase, SP2340, but underestimates the amount of *trans* isomers due to 18:1 positional isomer overlap. The combined GLC–IR method may circumvent this problem but at the cost of time, effort, and precision. *Trans* isomers in refined (deodorized or stripped) oils are different in type and levels from isomers in partially hydrogenated oils; their *trans* isomers are mono-*trans* trienoic and dienoic isomers, occurring at levels up to about 1–3%. GLC conditions for hydrogenated samples are often not suitable for refined oils because of overlap problems, but this time in the 18:3 region. Through careful selection of stationary phase and temperature program optimization (Drylab®GC), we have developed a single method that is suitable for hydrogenated, as well as refined, processed oils. The accuracy was checked with *cis* and *trans* fatty acid fractions isolated by silver-ion exchange high-performance liquid chromatography. The *trans* values obtained with the optimized method are in good agreement with the results obtained for the isolated fractions. We propose that recommended methods describe GLC conditions in terms of separation criteria rather than recommending only a fixed combination of stationary phase and temperature program.

JAOCS 73, 275–282 (1996).

KEY WORDS: Capillary GLC, *cis-trans*, deodorized oils, fatty acid methyl esters, geometrical isomers, hydrogenated oils, optimization, positional isomers, stationary phases.

Detailed fatty acid (FA) analysis, that separates the FA, not only on the basis of chainlength and double bonds (DB), but also shows the different geometrical and positional isomers, is becoming more important. The data obtained with this type of analysis are used for raw material specifications and intake control. Also, legislators often require more detailed product labeling according to certain groups of FA, e.g., total saturated FA (SAFA) and levels of essential FA. The latter aspect is related to the beneficial aspects for human health of the *cis* mono- and *cis-cis* methylene-interrupted polyunsaturated FA isomers (MUFA-*c* and PUFA-*cc*). The possible health effects of *trans* FA and their nutritional aspects are currently under discussion. The position of *trans* FA in food has recently been reviewed (1). *Trans* FA isomers are formed during partial hydrogenation and refining (deodorization or stripping) of vegetable oils, but the types and levels vary. Processing procedures are being modified to minimize the level of *trans* isomers. This variety requires simple and accurate analytical procedures. The methods should identify as many different isomers as possible, for both types of processed oils, at levels ranging from almost nil to as high as 50–60%.

In hydrogenated samples, the FA isomers are mainly monoenoic *cis* and *trans* positional isomers, formed by migration and conjugation of the DB systems. They show a typical distribution of the DB around the original positions with levels and isomer distributions depending on the hydrogenation conditions and degree of hydrogenation (2). The existing official [capillary gas–liquid chromatography (GLC)] methods on highly polar stationary phases are limited in their applicability for these samples due to overlap of certain *cis* and *trans* positional isomers. The Official AOCS Method (3) prescribes only one type of stationary phase, SP2340. Difficulties with quantitation of 18:1 isomers have been reported using SP2340 (3,4). *Trans* FA levels may be underestimated, with relative deviations up to 32% from the true value (5). A proposed combination of GLC and infrared (IR) techniques should overcome these problems in hydrogenated samples, but at the cost of a more laborious method with low precision, mainly determined by the IR method (6,7). Moreover, this combined GLC–IR method is recommended for *trans* levels above 5%.

The isomers that may be formed in vegetable oils during refining are quite different in type and levels from those formed during the hydrogenation process (8). Whereas in hydrogenated oils and fats the analytical focus is mainly on the monoenoic *cis* and *trans* FA, isomers formed during deodorization are mostly di- and trienoic mono-*trans* FA. This is related to the higher reactivity of the di- and trienoic FA in the formation of *trans* isomers. The DB do not shift in position, but only one DB isomer-

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izes from *cis* to *trans* (9,10,11). *Trans*-isomer formation during refining is temperature- and time-dependent, but under severe conditions, total levels up to 3.5% have been reported (12). For these oils, analytical methods have to focus on di- and trienoic mono-*trans* isomers at low levels. Because the official methods are not specifically designed for these types of oils, quantitation by GLC of the linolenic acid *trans* isomers formed during refining could result in inaccurate values due to their coelution with other FA [e.g., with *cis*-11 20:1 or linolenic acid in rapeseed oil (13)].

Suitable methods of FA quantitation for label claim purposes must be able to detect and to quantitate all groups of natural FA isomers, next to the *trans* isomers formed during hydrogenation or refining. For instance, recognized groups of FA allowed on product labels as mentioned in legislation are SAFA, MUFA-*c*, and PUFA-*cc*, AOCS Method Ce 1c-89 (3) could be used to quantitate these groups of FA, although with limitations on the accuracy of the *cis* and *trans* levels in hydrogenated oils. For refined oils, the method has limitations for MUFA-*c* and PUFA-*cc* levels due to overlap of the *cis*-11 20:1 and linolenic acid.

Preferably, a single capillary GLC method should give all information for both types of oils: (i) the detailed FA composition, allowing labeling of SAFA, MUFA-*c*, PUFA-*cc*, and total *trans* levels, requiring accurate levels of *cis* and *trans* monoenoic isomers in hydrogenated oils and (ii) *trans*-isomer levels in refined oils. Usually, methods for FA analysis prescribe strict combinations of stationary phases and conditions rather than defining suitability criteria in terms of required resolution of critical FA peak pairs. We tested the feasibility of defining suitability criteria by applying an optimization strategy to three highly polar cyanopropyl polysiloxane stationary phases (CP^{TM}-Sil 88, SP2340, BPX70), in the analysis of both hydrogenated and refined vegetable oil FA methyl ester (FAME) samples.

EXPERIMENTAL PROCEDURES

Samples and chemicals. Samples of vegetable oils, both nonhydrogenated (refined) and hydrogenated to various hydrogenation degrees, were obtained from several commercial sources. FAME (>99%) used for identification by the equivalent chainlength (ECL) concept (14) were purchased from Larodan Fine Chemicals AB (Malmö, Sweden) and Nu-Chek-Prep, Inc. (Elysian, MN). Certified reference material (CRM, code CRM No. 162, soy-maize oil blend) was obtained from the European Community Bureau of Reference (BCR, Brussels, Belgium) and used to check the quantitation, including the FAME preparation step.

Preparation of FAME. FAME were prepared from the oils and fats after alkaline hydrolysis, followed by methylation in methanol with BF_3 as catalyst (15). The final concentration of the FAME was approximately 7 mg/mL in heptane.

GLC. Analyses of FAME were carried out on a Chrompack CP9000 chromatograph, equipped with a Chrompack Autosampler-M911 (Chrompack, Middelburg, The Netherlands) for split-type injection, and a flame-ionization detector (FID). Fused-silica columns were used for the FAME analysis; CP^{TM}-Sil 88, 50 m × 0.25 mm i.d., 0.2 μm film (Chrompack); SP2340, 60 m × 0.25 mm i.d., 0.2 μm film (Supelchem, Leusden, The Netherlands), and BPX70, 50 m × 0.22 mm i.d., 0.25 μm film (SGE Inc., Austin, TX). Injection port and detector were kept at 250°C.

Silver-ion liquid chromatography (Ag-HPLC). FAME mixtures were separated into groups according to the number and geometry of the DB, by using silver-loaded ion-exchange columns (250 × 4.6 mm, Chromspher Lipids; Chrompack). Saturated and monoenoic FAME groups were eluted from the silver-loaded ion-exchange column with 0–22% linear gradient of acetone as modifier in dichloromethane/dichloroethane (50:50, vol/vol). The FAME groups were monitored with a Tremetrics 945 LC FID (LC-FID 945; Tremetrics, Austin). Fractions were collected with a standard laboratory fraction collector on time-base, bypassing the liquid chromatography–FID. For quantitation of the monoenoic *trans* fraction, the naturally present 18:0 FA was used as internal standard. A suitable nonnatural internal FAME standard (*cis* 17:1) was added to the samples before fractionation for quantitation of the monoenoic *cis* fraction.

RESULTS AND DISCUSSION

Suitability criteria for FA separations. The identification and quantitation of *cis* and *trans* isomers in the AOCS Recommended Method Ce 1c-89 (3) are based on the assumption that, following the prescribed combination of stationary phase (SP2340) and temperature program, all *cis* and *trans* positional 18:1 isomers are separated into two distinct peak groups. However, comparison of total sample analysis with GLC chromatograms of the isolated *trans* and *cis* 18:1 isomers shows the coelution of specific *trans* and *cis* isomers (Fig. 1). The *trans* 18:1 isomers with DB in the 12- and 13-positions coelute with the *cis* isomers (carbon atoms are numbered starting from the carbonyl moiety). *Trans* isomers with DB in higher positions, e.g., 14 and 15, possibly formed during hydrogenation processes, will also coelute with the *cis* isomers. This effect was studied in detail for several oil types. The incomplete resolution of the *trans*-12 and *trans*-13 18:1 isomers, the so-called valley peaks, resulted in an underestimation of the *trans* level by as much as 12% at a total *trans* level of 50% (absolute values).

The choice of the stationary phase strongly influences resolution. Quantitation of the *cis/trans* isomer levels with the column prescribed in AOCS Method Ce 1c-89 (SP2340) (3) or, for example, a separation on CP^{TM}-Sil 88, has a marked effect on the observed *trans* levels, although both columns are marketed as highly polar cyanopropyl polysiloxane phases. Table 1 compares results obtained by analysis of some partially hydrogenated vegetable oil samples with the CP^{TM}-Sil 88 column (temperature program from 140 to 180°C at 8°C/min; used until recently in our laboratory for *cis/trans* 18:1 isomer separations) and those obtained by AOCS

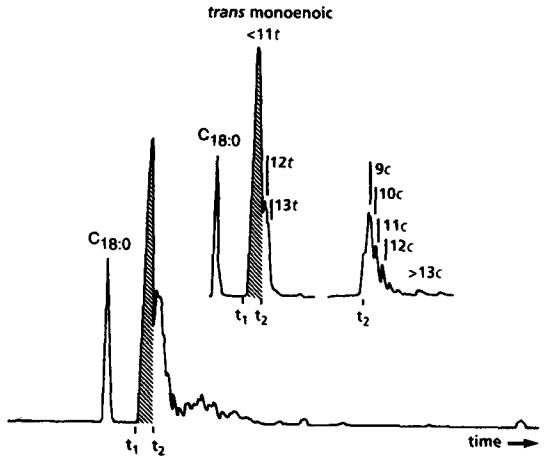

FIG. 1. Chromatogram on SP2340 (Supelchem, Leusden, The Netherlands), column (AOCS Method Ce 1c-89, Ref. 3) of a partially hydrogenated soybean sample (hBO2). The total sample chromatogram and the chromatogram of the isolated *cis* and *trans* isomers are shown for the 18:0 and 18:1 retention area only. *Cis* and *trans* isomers were isolated *via* silver-ion liquid chromatography. The shaded areas in the total chromatogram and the fractions indicate the areas that would be assigned as *trans*. Start (t_1) and end times (t_2) used for integration of the 18:1 *trans* area, are indicated.

TABLE 1
Results of *trans* 18:1 Values as Obtained with AOCS Method (SP2340)[a], by the CP™-sil 88 Method[b], and by Analysis of the Isolated *trans* Fraction, Obtained *via* Ag-HPLC[c]

Sample type	AOCS Official Method Ce 1c-89	In-house method, CPTMSil-88	Result *via* Ag-HPLC fractionation and internal standard
hBO1	10.0	12.6	12.7
hBO2	37.8	50.5	47.9
hBO3	31.8	46.0	43.2
hRP	11.9	14.3	14.9

[a]Reference 3. SP2340 from Supelchem (Leusden, The Netherlands).
[b]CP™-Sil 88 from Chrompack (Middelburg, The Netherlands). See text for details of method.
[c]Silver-ion liquid chromatography.

Method Ce 1c-89 (3) with results obtained after fractionation of the *trans* isomers by Ag-HPLC. The latter reflects the true *trans* 18:1 isomer level.

Although both methods were developed for *cis/trans* 18:1 separations, the *trans* levels obtained with the CP™-Sil 88 stationary phase (vs. SP2340) are closer to the values obtained with isolated fractions. Also, this proves again that the two valley peaks are mainly *trans* isomers: *trans*-12 and *trans-13* 18:1. Still, a small error in the *trans* GLC level could be observed with the CP™-Sil 88 column due to small amounts of *cis* isomers (i.e., *cis*-6 to *cis*-8 18:1 isomers) coeluting with the *trans*-12 and *trans*-13 isomers.

To quantitate as accurately as possible the *trans* and *cis* 18:1 isomer levels, conditions have to be selected to separate the *trans* isomers with the DB on the 12- and 13-carbon atoms. These *trans*-12 and *trans*-13 18:1 isomers are recognized as the one or two valley peaks that elute between the *trans*-8 to *trans*-11 18:1 peak group and the *cis*-9 18:1 isomer. This allows accurate integration of the *trans* area, ending with the *trans*-13 isomer peak. So, the first requirement for optimal separation and quantitation is the separation between the *trans*-13 18:1 and *cis*-9 18:1 isomers.

Next to separation and quantitation of the *cis* and *trans* 18:1 isomers, it would be desirable if the analytical method could also quantitate all other FA in the samples because the results could then be used to specify the levels of SAFA, MUFA-*c*, and PUFA-*cc* on the product label. When the AOCS method is used, the naturally present isomers α-linolenic acid (*cis*-9,*cis*-12,*cis*-15 18:3; now referred to as *ccc*-18:3) and *cis*-11 20:1 coelute. This separation is especially difficult for samples that contain relatively high levels of *cis*-11 20:1, e.g., rapeseed oil. The coelution of 18:3 isomers and *cis*-11 20:1 is also observed with the CP™-Sil 88 stationary phase. We have defined this criterion, the identification of the label claim fatty acids *cis* 20:1 and *ccc*-18:3, not disturbed by coelution of *trans* 18:3 isomers, as a second requirement in FA isomer analysis. A new, single GLC method for hydrogenated and refined oils should comply with the following requirements: (i) maximum resolution between the *trans* and *cis* isomers in the 18:1 area. More specifically, the *trans*-13 18:1 isomer must be separated from the *cis*-9 18:1 isomer (requirement 1). (ii) 20:1 should elute, with sufficient resolution from *ccc*-18:3, to allow correct integration. Elution of *cis*-11 20:1 after the linolenic acid peak is ideal (requirement 2a). If this is not possible, elution of 20:1 between the last eluting *mono-trans* 18:3 isomer (*trans*-9,*cis*-12,*cis*-15) and *ccc*-18:3 is then the alternative (requirement 2b).

Optimization procedure. The elution order in capillary GLC of *cis* and *trans* monoenoic isomers can be influenced by modifying the temperature conditions. Based on only two temperature gradient runs, the retention times (Rt) at other temperature conditions can be simulated with suitable software, e.g., Drylab®GC (revision 1.10; LC-Resources Inc., Walnut Creek, CA). The Rt of the specific isomers are input in the simulation program, together with general column and GLC information. The resolution between the *trans*-13 18:1 and *cis*-9 18:1 isomers and the resolution between the *cis*-11 20:1 and 18:3 isomers can be assessed for requirements 1, 2a, and 2b at various simulated conditions. For practical reasons, the simulated shifts in Rt, at different temperatures, are expressed relative to the 18:0 Rt as delta relative retention times (DRRt). DRRt for two peaks *a* and *b* is defined as DRRt = $(Rt_a - Rt_b)/Rt_{18:0}$. This allows visualization of the three relevant DRRt values on the same scale for a range of simulated isothermal conditions. The defined criterion is met if: DRRt >0.01 for requirement 1, and the DRRt value should either be below zero (*cis*-11 20:1 elutes after *ccc*-18:3) for criterion 2a, or the DRRt value should be above zero (*cis*-11 20:1 elutes before *ccc*-18:3 but after the *tcc*-18:3 isomer), requirement 2b. The limit value of DRRt > 0.01 for requirement 1 was determined from a real chromatogram.

Optimization results and validation. The simulation results obtained for the CP™-Sil 88 stationary phase column are presented in Figure 2 (solid lines) with some experimental data.

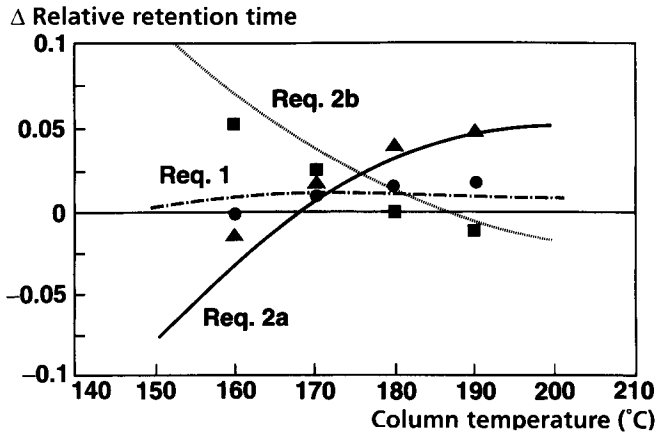

FIG. 2. Window diagram obtained from the simulated separations at isothermal conditions for the CP™-Sil 88 (Chrompack, Middelburg, The Netherlands) stationary phase. The y-axis represents delta relative retention time (DRRt), relative to 18:0. An absolute DRRt value of 0.01 or larger indicates that peaks are clearly distinguishable. Requirement (Req.) 1 describes the separation between the *trans*-13 18:1 and the *cis*-9 18:1 isomer. Req. 2a describes the separation between the *cis*-11 20:1 isomer and the *ccc*-18:3 peak, a negative DRRt value indicates elution of 20:1 after *ccc*-18:3. Req. 2b describes the separation between the 18:3 *tcc*-isomer and the *cis*-11 20:1 peak. Lines indicate simulated values. Experimental data points are plotted in the figure; 1 = ●, 2a = ▲, and 2b = ■.

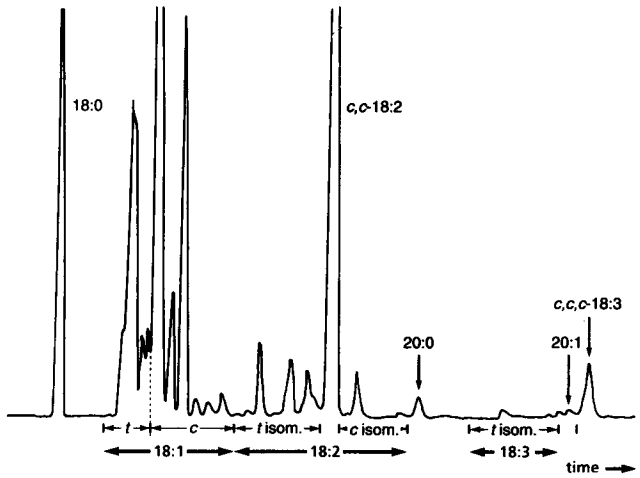

FIG. 3. Example chromatogram of sample hBO1, showing the peak-labeling and ranges used for isomer (isom.) quantitation. Conditions: 175°C isothermal on CP™Sil-88 stationary phase. Source as in Figure 2.

Requirement 2a is met up to 165°C, but unfortunately requirement 1 is not. Hence, the condition for which the *cis*-11 20:1 peak elutes between *tcc*-18:3 and *ccc*-18:3 (requirement 2b) remains the alternative. This condition is predicted in the window diagram at 176°C for the CP™-Sil 88 stationary phase column. The crossing of the two lines 2a and 2b indicates positioning of the 20:1 peak exactly between the *tcc*-18:3 and *ccc*-18:3 isomers. At this simulated condition, the separation is still good enough to separate *trans*-13 18:1 from *cis*-9 18:1 (DRRt above 0.01). Fine-tuning of the predicted condition with real elutions resulted in an optimum at 175°C. The minute differences in separation at 176 and 175°C are only visible upon careful inspection of the chromatograms. The temperature conditions, obtained from simulation and fine-tuning with real experiments, were validated with partially hydrogenated and refined samples and isolated fractions of *cis* and *trans* isomers. The identification of the different FA groups in the resulting chromatograms is based on the ECL values for individual FA or ECL ranges for isomers, e.g., 18:2 isomers (Fig. 3). The results obtained with this method show that a good reproducibility is obtained, allowing accurate and precise reporting of FA levels (Table 2).

The same approach can also be used to optimize a gradient temperature program that meets the same requirements. For CP™-Sil 88 stationary phase, the predicted gradient

TABLE 2
Reproducibility of 18:1 Isomer Separations[a]

Component	Sample			
	hBO1	hBO2	hRP	RP refined
18:0	7.37 ± 0.02	9.61 ± 0.03	7.86 ± 0.03	1.54 ± 0.01
trans 18:1 isomers	12.62 ± 0.14	50.16 ± 0.12	14.34 ± 0.06	0.03 ± 0.01
cis 18:1 isomers	35.58 ± 0.02	16.90 ± 0.11	47.89 ± 0.09	57.88 ± 0.07
trans 18:2 isomers	4.18 ± 0.15	9.59 ± 0.07	5.13 ± 0.05	0.17 ± 0.02
cc 18:2 (linoleic)	25.30 ± 0.06	0.55 ± 0.04	13.02 ± 0.02	20.65 ± 0.03
cis 18:2 isomers	1.14 ± 0.02	0.70 ± 0.08	1.07 ± 0.03	ND[b]
trans 18:3 isomers	0.55 ± 0.03	0.06 ± 0.02	0.63 ± 0.06	1.23 ± 0.04
ccc-18:3 (linolenic)	1.29 ± 0.04	0.02 ± 0.03	1.37 ± 0.02	10.07 ± 0.06
cis-11 20:1	0.21 ± 0.01	0.01 ± 0.02	0.95 ± 0.01	1.50 ± 0.02
Sum level of *trans* isomers (gas–liquid chromatography)	17.35 ± 0.16	59.81 ± 0.14	20.10 ± 0.10	1.43 ± 0.05
Total *trans* level obtained *via* infrared method (single determination)	18	63	21	—

[a]Column: CP™-Sil 88 (Chrompack, Middelburg, The Netherlands) isothermal condition 175°C. Samples were analyzed on three different days, duplicates on each day, analyses were performed in random order. Results are expressed as mean observed values and the standard deviation (SDn-1). The total *trans* level as obtained with infrared spectroscopy is given for comparison.

[b]ND = not detected, < 0.01%.

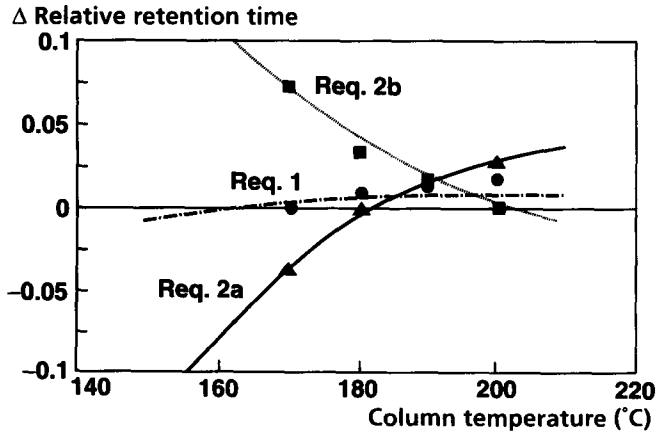

FIG. 4. Window diagram obtained from the simulated separations under isothermal conditions for the SP2340 stationary phase. Experimental data points are plotted in the figure. See Figure 2 for symbols and abbreviation; see Figure 1 for source.

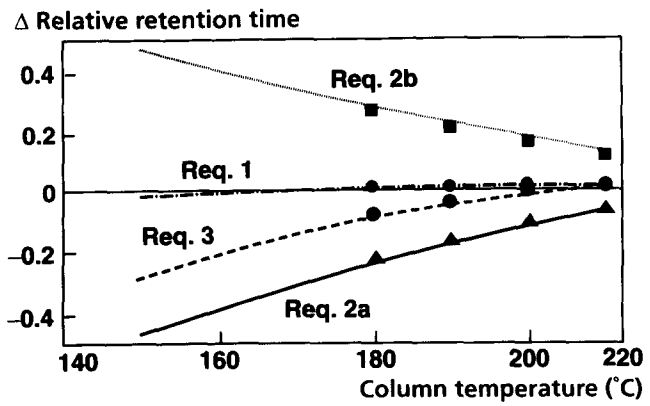

FIG. 5. Window diagram obtained from the simulated separations under isothermal conditions for the BPX70 (SGE Inc., Austin, TX) stationary phase. Requirement 3 describes the simulated behavior of the 20:0 peak. Experimental data points are plotted in the figure. See Figure 2 for symbols and abbreviation.

(140°C, slope 8°C/min, final 176°C) was similar to the one found earlier empirically. Both situations, isothermal and temperature gradient, allowed identification of FA starting from 6:0 and higher. No improvement in run time or range of FA (in chainlength) could be found between the optimum gradient and isothermal conditions. The performance of both methods at the predicted isothermal and gradient conditions for the CP™-Sil 88 stationary phase was tested with real samples and *trans* fractions. No statistically significant difference could be found in *trans* values between the isothermal and gradient programs. Because temperature gradients show no practical improvements, we prefer the isothermal condition, the latter allowing the more accurate determination of ECL for FA identification.

The same criteria were used to optimize the separations on a SP2340 stationary phase. The simulation diagram again predicts an optimum, now at 192°C, which was verified experimentally (Fig. 4). The somewhat different properties of the BPX70 column resulted in elution of the *cis*-11 20:1 after the *ccc*-18:3 isomer over the whole range of simulated temperatures (Fig. 5). This would indicate that, contrary to the other two tested phases, requirement 2a can be met in all situations. There is no sharply defined optimum in the BPX70 window diagram, indicated by a crossing of the lines. Above approximately 195°C, requirement 1 is met (DRRt > 0.01) and still increases with the temperature. But, at temperatures above 200°C, the 20:0 peak starts to interfere with *ccc*-18:3. This "new" problem could be solved by plotting the 20:0 DRRt values in the same window diagram (requirement 3). The predicted isothermal condition for which the 18:1 isomer separation is optimal and the 20:0 peak does not interfere is found at 198°C. Also, the different properties of the BPX70 phase resulted in a shift of some of the *trans* 18:2 isomers toward the group of *cis* 18:1 isomers. This could result in slightly overestimating the *cis* 18:1 amount in samples that contain high amounts of 18:2 isomers, and consequently in an underestimation of the 18:2 *trans* isomers.

The final temperature conditions for all columns, obtained

TABLE 3
Gas–Liquid Chromatography Conditions Used in the Experiments[a]

	Method			
	AOCS Official Method Ce 1c-89	Proposed optimum condition for SP2340	Proposed optimum condition for CP™-Sil 88	Proposed optimum condition for BPX 70
Stationary phase and column dimensions	SP2340 Column, 60 m × 0.25 mm i.d. × 0.2 µm film	SP2340 60 m × 0.25 mm i.d. × 0.2 µm film	CP™-Sil 88 50 m × 0.25 mm i.d. × 0.2 µm film	BPX70 50 m × 0.22 mm i.d. × 0.25 µm film
Temperature conditions	Start 150°C, slope 1.3°C/min, final 200°C, hold 10 min	Isothermal 192°C	Isothermal 175°C	Isothermal 198°C
Column head pressure (kPa)	125	125	130	155
Linear velocity carrier (helium)	16 cm/s	15 cm/s	19 cm/s	17 cm/s
Injection volume (µL), split ratio	0.7 1:100	0.7 1:100	0.7 1:100	0.7 1:100
Injector, detector temperature (°C)	250, 250	250, 250	250, 250	250, 250

[a]See Table 1 for source locations and method information. BPX70, from SGE, Inc. (Austin, TX).

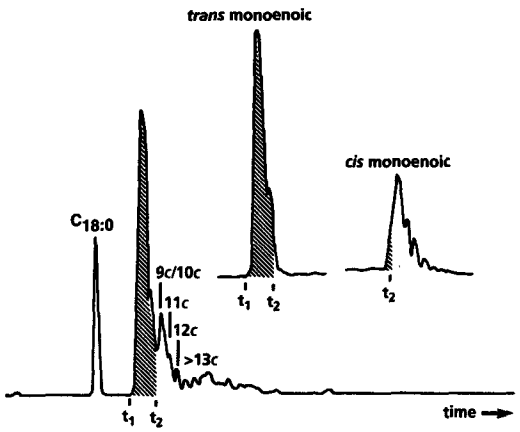

FIG. 6. Chromatogram showing 18:1 isomer separation at optimum conditions on the SP2340 phase. The shaded areas in the total chromatogram and the fractions indicate the areas that would be assigned as *trans*. Sample hBO2, source as in Figure 1.

from the window diagrams and the fine-tuning experiments, are presented in Table 3, together with the instrument settings. Elution for 22:0 is approximately 25 min for all three columns under these conditions. We used the optimized methods for samples with *trans* levels ranging from almost zero to approximately 65%. Example chromatograms, showing the 18:1 retention area of the samples analyzed with the SP2340, the CP^{TM}-Sil 88, and the BPX70 columns, are shown in Figures 6, 7, and 8, respectively. Separation of the 20:0, *cis*-11 20:1, *ccc*-18:3, and the *tcc*-18:3 isomer peaks in refined oil is shown in Figure 9 for all stationary phases investigated.

The combined information on elution patterns of the different *cis* and *trans* positional isomers is summarized in Figure 10. Based on the information from the window diagrams, the same optimized separations can be obtained, starting at the indicated temperatures, by only varying the initial temperature by a few degrees.

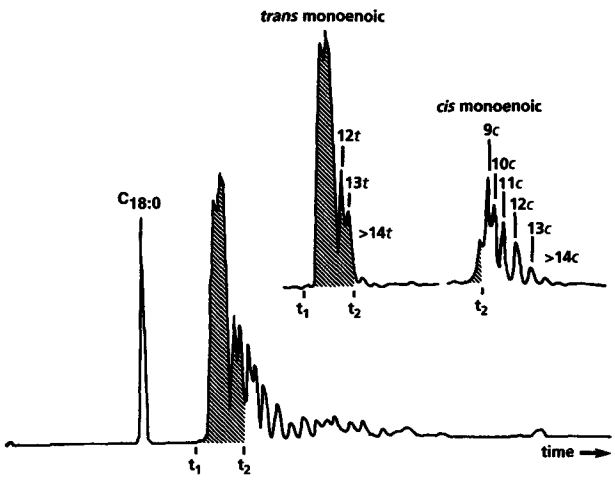

FIG. 7. Chromatogram showing 18:1 isomer separation at optimum conditions on the CP^{TM}-Sil 88 phase. The shaded areas in the total chromatogram and the fractions indicate the areas that would be assigned as *trans*. Sample hBO2, source as in Figure 2.

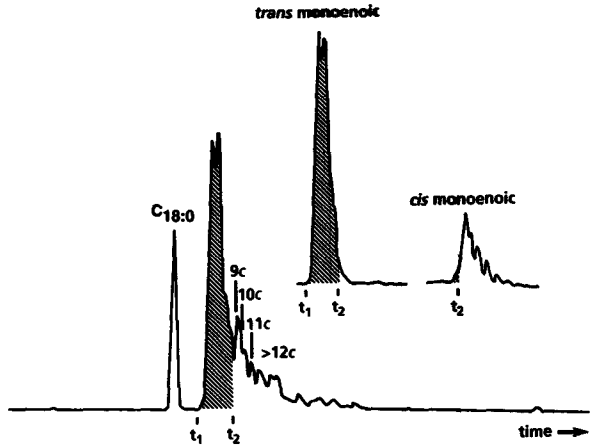

FIG. 8. Chromatogram showing 18:1 isomer separation at optimum condition on the BPX70 phase. The shaded areas in the total chromatogram and the fractions indicate the areas that would be assigned as *trans*. Sample hBO2, source as in Figure 5.

To check on the precision of the method, several samples were analyzed on different days on the three stationary phases under optimum conditions (Table 4 and Fig. 11). The samples represent different 18:1 levels and isomer distributions. The results show that for all three phases the obtained levels of *cis* and *trans* are in good agreement. Moreover, the *trans* values agree with results obtained for fractions isolated by Ag-HPLC (considered to be true values). Small differences between the result of the isolated *cis* fractions and the overall analyses are explained by the experimental error made in the quantitation of the isolated *cis*-fraction (I.S. addition) and the small overlap in the total chromatogram.

As reported previously, the *trans* values obtained with the SP2340 stationary phase by the AOCS method (3) are too low. This effect is completely eliminated under the described optimized isothermal conditions. For the BPX70 column, the *cis*

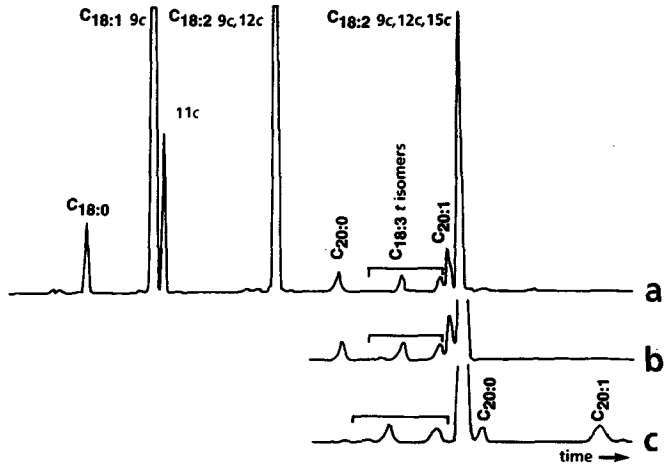

FIG. 9. Chromatograms of a refined rapeseed oil sample (RPref), focusing on the 20:1/18:3 retention area. The results of the three different stationary phases are plotted in one figure for comparison. Legend: a = CP^{TM}-Sil 88, isothermal 175°C; b = SP2340, isothermal 192°C, and c = BPX70, isothermal 198°C. See Figures 1, 2, and 5 for source information.

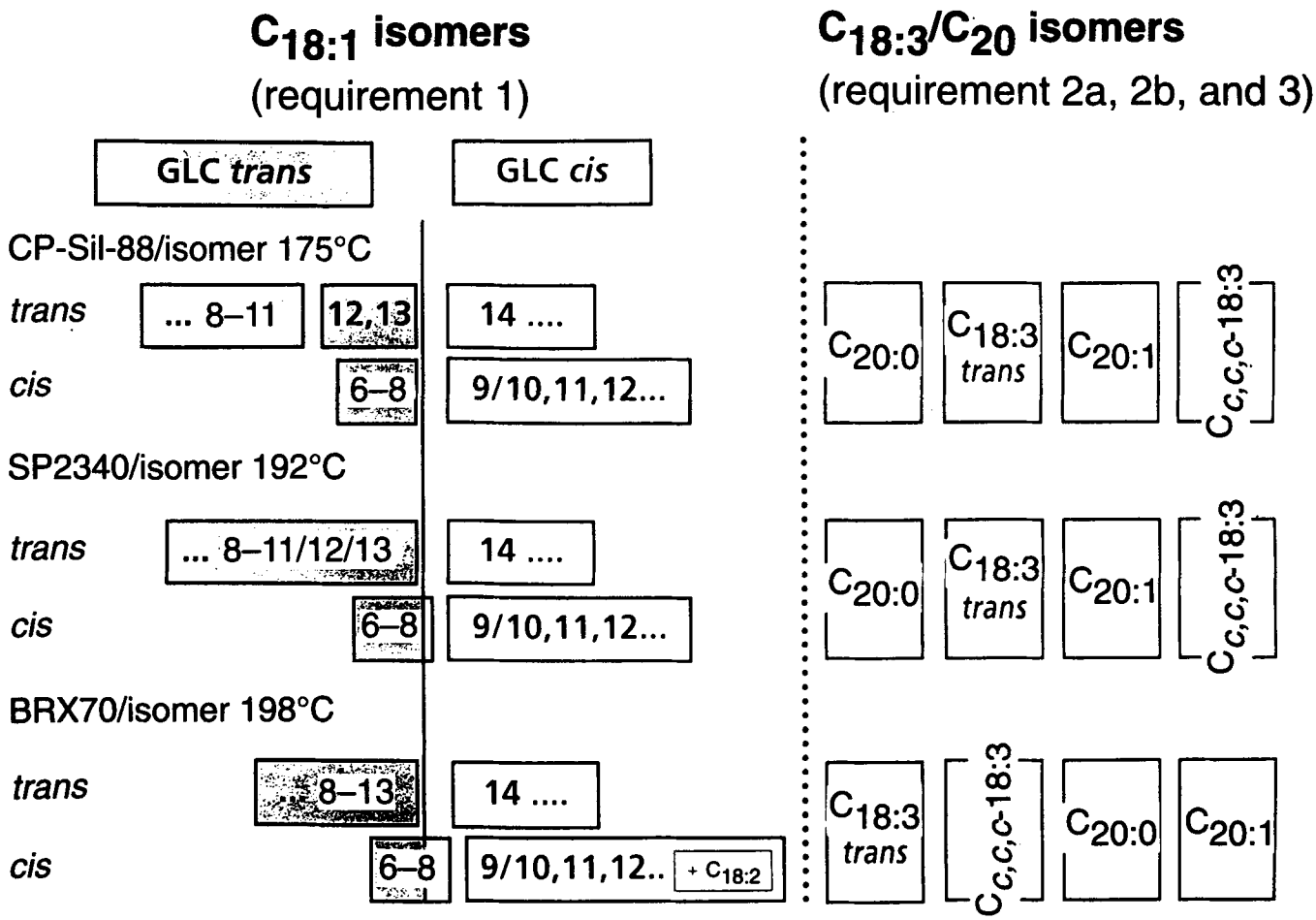

FIG. 10. Schematic representation of the different positional fatty acid isomer distributions, obtained at optimum isothermal temperature conditions for three stationary phases. The vertical line represents the peak split, which would be made between the *trans* 18:1 (shaded) and *cis* 18:1 peak groups for the quantitation. The elution order of the *tcc*-18:3, 20:0, *cis*-20:1, and *ccc*-18:3 isomers are indicated on the right side of the diagram. GLC, gas–liquid chromatography; see Figures 1, 2, and 5 for source information.

level found in the hBO2 sample is higher than that for the two other columns. This can be explained by the presence of relatively high amounts of 18:2 isomers, which partly coelute with the *cis* 18:1 isomers.

The proposed optimization method for the determination of FA isomers in partially hydrogenated and refined oils and fats yields isothermal conditions that produce accurate and precise results. Monoenoic *cis* and *trans* isomers are quantitated with good accuracy. Di- and trienoic isomers are well separated, allowing the determination of PUFA-*cc* for label claims.

Our results show that, when defining normative methods, the separation should preferably be described in terms of separation requirements, rather than recommending a fixed combination of stationary phase and conditions. For critical separations, deviations between instrument settings and real conditions might ruin the separation. The suitability criteria for

TABLE 4
***Cis* and *trans* 18:1 Fatty Acid Levels as Determined by Several Methods on Different Stationary Phases[a]**

	Column/method									
	SP2340 AOCS Official method Ce 1c-89		CP™-Sil 88, isothermal 175°C		SP2340, isothermal 192°C		BPX70, isothermal 198°C		*via* Ag-HPLC fractions	
Samples	18:1 *cis*	18:1 *trans*	18:1 *cis*	18:1 *trans*	18:1 *cis*	18:1 *trans*	18:1 *cis*	18:1 *trans*	18:1 *cis*	18:1 *trans*
hBO1	37.8	10.0	35.6	12.6	35.7	12.5	36.0	12.5	33.4	12.7
hBO2	28.4	38.4	16.9	50.2	18.1	49.3	23.1	49.1	17.5	47.9
hRP	50.0	11.9	47.9	14.3	47.9	14.2	49.1	14.1	48.3	14.9

[a]Mean values are reported only. Standard deviations of the reported results are in the range of 0.02 to 0.06 (refer to Table 2). Sources and method information as in Tables 1 and 3.

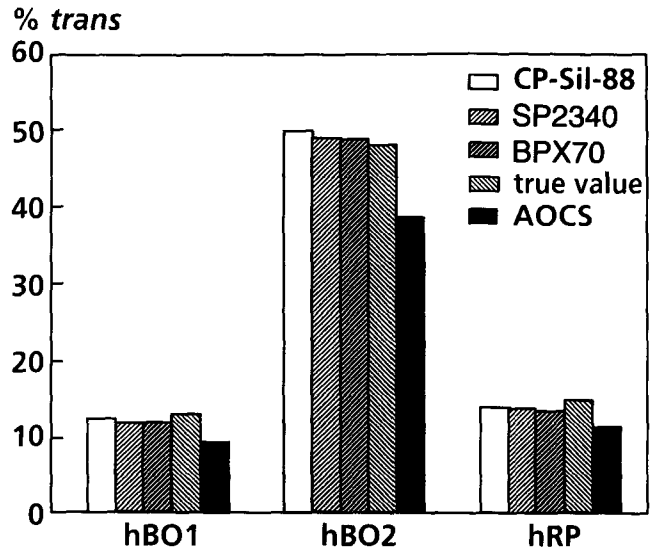

FIG. 11. Bar chart of the *trans* values of three different samples, with different levels and isomer distributions. The results, as obtained with the optimized methods, are compared with those of the AOCS Official Method (Ref. 3) and the *trans* levels calculated from isolated fractions. See Figures 1, 2, and 5 for source information.

detailed FA isomer analyses are: (i) 18:1 "valley peaks" should be well separated from the *cis*-9 18:1 isomer and (ii) in the 18:3 area, the peak of *cis*-11 20:1 should not overlap that of *ccc*-18:3 or the *tcc*-18:3 isomer.

Using the proposed optimization strategy, a single method can be developed for the analysis of hydrogenated and refined oils. The optimization strategy is suitable for at least three different stationary phases. The found optimum conditions for the three tested phases would require only small temperature corrections, if any, due to instrument and/or column batch differences. So far, replacement of the CP^{TM}-Sil 88 column required only one-degree corrections to handle column batch differences, e.g., 174°C instead of 175°C.

The resulting capillary GLC method is preferred to the current AOCS procedure or the combined GLC–IR procedure for the analyses of *trans* levels and for label claim purposes. By careful selection of stationary phase and optimizing the temperature conditions, the method reports more accurate total *trans* contents at low levels and without overlap between *cis*-11 20:1 and linolenic acid or its *mono-trans* isomers. Our procedure also avoids the use of two different GLC methods, each with its own application, i.e., for hydrogenated and refined oils.

ACKNOWLEDGMENT

The authors acknowledge their gratitude to J.R. Klooster for preparation of the isolated FAME groups by Ag-HPLC.

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[Received May 1, 1995; accepted November 28, 1995]