Determination of *cis* and *trans*-Octadecenoic Acids in Margarines by Gas Liquid Chromatography-Infrared Spectrophotometry[1]

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A combined capillary gas liquid chromatography (GLC) and infrared spectrophotometry (IR) method is described for the determination of *cis* and *trans*-octadecenoic acids in margarines made from partially hydrogenated vegetable oils. The total *trans*-unsaturation of margarine fatty acid methyl esters determined by IR, with methyl elaidate as the external standard, was correlated to the capillary GLC weight percentages of the component *trans* fatty acid methyl esters by the mathematical formula:

IR *trans* = %18:1*t* + 0.84 × %18.2*t* + 1.74 × %18:2*tt* + 0.84 × %18:3*t* where 0.84, 1.74 and 0.84 are the correction factors which relate the GLC weight percentages to the IR *trans*-equivalents for mono-*trans*-octadecadienoic (18:2*t*), *trans, trans*-octadecadienoic (18:2*tt*) and mono-*trans*-octadecatrienoic (18:3*t*) acids, respectively. This formula forms the basis for the determination of total *trans*- and *cis*-octadecenoic acids in partially hydrogenated vegetable oils. From the weight percentages of 18:2*t*, 18:2*tt* and 18:3*t* determined by capillary GLC on a cyanosilicone liquid phase and the total *trans*-unsaturation by IR, the percentage of the total *trans*-octadecenoic acids (18:1*t*) is calculated using the formula. The difference between the total octadecenoic acids (18:1), determined by capillary GLC, and the 18:1*t* gives the total *cis*-octadecenoic acids.

KEY WORDS: *cis* and *trans*-Octadecenoates, *cis-trans* fatty acid isomers, GLC-IR analysis, hydrogenated fat, margarine.

Margarines and other dietary fats made from partially hydrogenated vegetable oils contain substantial amounts of *trans*- and *cis*-octadecenoic acids (1-5). Accurate determination of the total *cis* and *trans*-octadecenoic isomers is a difficult task, as there is no one-step, simple procedure for the separation of the *trans*-octadecenoic acids (18:1*t*) as a group from that of the *cis* (18:1*c*) isomers (6-9). Direct analysis by gas liquid chromatography (GLC) on the very polar cyanosilicone liquid phases has been recommended for *cis-trans* isomeric separations (10-13). The direct GLC method is uncomplicated and straight forward and, therefore, it has been the method of choice in most laboratories specializing in oils and fats. However, a complete resolution of isomeric octadecenoic acids (18:1) is not feasible by GLC alone (5-9, 14-16). In most polar columns, the *trans* group of isomers elutes after 18:0, but the later eluting *trans* isomers (the high Δ value isomers such as 18:1Δ12-14) are under the major 18:1Δ9*c* component (5,16; also see Fig. 1). In spite of the *cis-trans* overlaps, many workers considered the major 18:1 peak in GLC to be 100% *cis* (10-13). The 18:1*t* content as determined by GLC alone is too low by several percentage points in favor of the respective 18:1*c* (16). Sampugna *et al.* (15) proposed the use of appropriate correction factors to compensate for the *cis-trans* overlaps. The correction varies with the ratio of 18:1*c* to 18:1*t* in the fat sample. It is therefore necessary to set up a series of correction factors for various ratios of 18:1*c* to 18:1*t* with authentic standards. GLC combined with other chromatographic techniques (particularly argentation chromatography) has been suggested (6,8,9,16), but these procedures are lengthy and are not suitable for routine analysis of dietary fats.

trans-Double bonds in aliphatic components absorb at approximately 10.3 μ in the infrared (IR) region. Measurement of the intensity of this absorption is the basis of the official methods (17-19) for the determination of total *trans*-unsaturation in fats. The *trans*-unsaturation measured by IR is a function of the various *trans* components present in the fat sample. In this paper we propose a simple mathematical formula relating the total *trans* unsaturation, determined by IR, to the weight percentages of the component *trans* fatty acids in the margarine sample measured by GLC on a cyanosilicone liquid phase. The proposed formula forms the basis for the determination of total 18:1*t* and finally the total 18:1*c* in margarines and in other partially hydrogenated vegetable oils.

EXPERIMENTAL PROCEDURES

Methyl esters of stearic (18:0), elaidic (18:1Δ9*t*), linoleic (18:2Δ9*c*, 12*c*) and linoelaidic (18:2Δ9*t*, 12*t*) were purchased from the Sigma Chemical Company (St. Louis, MO). Commercial margarines were purchased from supermarkets in Ottawa, Canada between January and April, 1989. A mixture of *cis*-9, *trans*-12-and *trans*-9, *cis*-12-octadecadienoic acids was prepared from linoleic acid via *p*-toluenesulfinic acid geometrical isomerization (20) and subsequent isolation of the mono-*trans*-diethylenic fraction through silver nitrate thin-layer chromatography ($AgNO_3$-TLC). The details of the procedures have been described elsewhere (16,20,21). The isolated mono-*trans* diethylenic fraction contained 45.8% *cis*-9, *trans*-12 and 54.2% *trans*-9, *cis*-12 isomers by GLC analysis.

Fat from margarine was extracted with methylene chloride, washed with water, dried over sodium sulfate and the solvent was evaporated to dryness. The extracted fat was converted into fatty acid methyl esters (FAME) according to the AOCS official method Ce 2-66 (19). For identification of margarine fatty acids, the FAME mixture initially was fractionated according to

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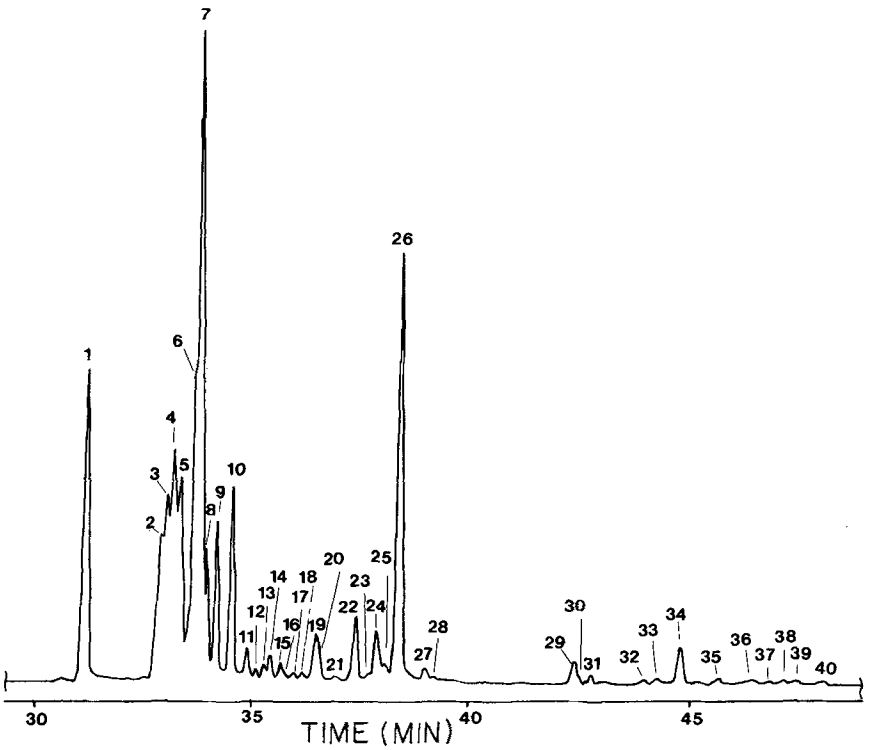

FIG. 1. The C_{18} region of the gas chromatogram of the fatty acid methyl esters of soybean oil margarine. Analysis on a SP-2560 flexible fused capillary column (100 m × 0.25 mm i.d.). Numbers correspond to 1, 18:0; 2, 18:1Δ6-8t; 3, 18:1Δ9t; 4, 18:1Δ10t; 5, 18:1Δ11t; 6, 18:1Δ12t; 7, 18:1Δ9c + 18:1Δ13t + 18:1Δ14t; 8, 18:1Δ10c; 9, 18:1Δ11c; 10, 18:1Δ12c + 18:1Δ15t; 11, 18:1Δ13c; 12, 18:2tt; 13, 18:1Δ14c; 14, 18:2tt; 15, 18:1Δ15c; 16, 18:2tt; 17,18, 18:2tc/ct; 19, 18:2Δ9t,12t; 20,21, non-methylene interrupted 18:2tc/ct; 22, 18:2Δ9c,12t; 23, ?; 24, 18:2Δ9t,12c; 25, ?; 26, 18:2Δ9c,12c; 27, 18:2Δ9c,15c; 28, ?; 29, 20:0; 30, 18:3?; 31, 18:3Δ9c,12c,15t; 32, 18:3Δ9t,12c,15c; 33, 18:3Δ9c,12t,15c; 34, 18:3Δ9c,12c,15c, 35, 20:1; 36-40, 18:2 conjugated ?.

the degree of unsaturation through the methoxy-bromomercuric (MBM) adducts. The MBM-adducts preparation and subsequent fractionation of the adducts by thin-layer chromatography (TLC) were carried out according to the procedure of Sebedio *et al.* (9). The recovered mono-, di- and triethylenic FAME fractions were further fractionated according to the geometry of the double bond(s) by $AgNO_3$-TLC. The various FAME fractions isolated were analyzed by GLC. The FAME of these fractions and the starting margarines were identified or characterized by comparing the GLC retention times with authentic standards, comparing the elution patterns or equivalent chain lengths (ECL) on columns of similar polarity published in the literature (22-24) and through GLC ECL calculations. The relative positions of the MBM-adducts on TLC and the FAME on $AgNo_3$-TLC further helped to established their identifications. A representative GLC profile of a margarine sample on a SP-2560 flexible fused silica capillary column (FFSC) is shown in Figure 1. The procedures mentioned above, the relevant literature and the fatty acid profiles of some margarines, including their GLC retention times on a SP-2340 FFSC, have been documented elsewhere (16).

GLC analyses of FAME were performed on a Varian Vista 6000 Gas Chromatograph (Varian Associates, Palo Alto, CA) equipped with a flame ionization detector. The GLC column was either a SP-2340 (60 m × 0.25 mm i.d., μm film thickness) or SP-2560 (100 m × 0.25 mm i.d., 20 μm film thickness) FFSC (both columns were purchased from Supelco, Inc. Bellefonte, PA). The SP-2340 column temperature was operated isothermally at 165°C. The SP-2560 column temperature was programmed at a rate

of 1°C/min from 125 to 175°C and held at that temperature for 25 min. With both columns the detector and the injection port temperatures were 235 and 225°C, respectively, and the hydrogen carrier gas pressure was 20 psig.

The $AgNO_3$-TLC analyses were performed on precoated Whatman K5 silica gel TLC plates (20 cm × 20 cm, 250 μm layer, Whatman International Ltd., Madison, England) impregnated with $AgNO_3$ by plates that were immersed in a 10% (w/v) solution of $AgNO_3$ in acetonitrile, and then dried horizontally and activated at 110°C for 1 hr. The plates were developed in chloroform containing 0.75% ethanol. The bands were visualized under UV light after spraying with an ethanolic solution of 2′, 7′-dichlorofluorescein (0.2%, w/v). The separated bands were scraped from the plate, extracted with hexane:chloroform (1:1, v/v) and concentrated for GLC analysis.

The total isolated *trans* unsaturation in the methyl esters of the margarine samples and in the various mixtures of *trans* fatty acid methyl ester standards with methyl stearate were determined according to the AOCS infrared spectrophotometric method (official method Cd 14-61) using methyl elaidate as the external standard (19). The IR analyses were performed on a Beckman IR 4230 Spectrophotometer (Beckman Instruments, Inc., Irvine, CA).

RESULTS AND DISCUSSION

The AOCS method (19), as well as the other official methods (17,18) calibrate *trans*-ethylenic absorption against methyl elaidate, the pure *trans*-9-octadecenoate; these methods are specific for this type of unsaturation only. There will be some of this fatty acid in partially hydrogenated vegetable oils, but also a wide variety of positional isomers and *trans* polyunsaturated fatty acids, all of which have slightly different IR absorption characteristics, contributing to the total *trans* content. Therefore, to relate the IR and GLC total *trans* unsaturation mathematically, it is necessary to know i) the component *trans* fatty acids in the sample; and ii) the correlation between the IR and GLC responses for each of those component *trans* fatty acids—in other words, the correction factors for relating the IR and GLC responses for the individual component *trans* fatty acids in the sample.

trans Fatty acids in margarine. The principal *trans* fatty acid group in margarine and other dietary fats made from partially hydrogenated vegetable oils is 18:1*t*. *trans* Isomers of 18:2 are always present in hydrogenated fats at low to moderate levels. Some of the margarines contained as much as 5-6% 18:2 *trans* isomers (5,16,25). The major diethylenic *trans* acids in partially hydrogenated vegetable oils have been well characterized as *cis*-9, *trans*-12-octadecadienoic (18:2Δ9*c*,12*t*), *trans*-9, *cis*-12-octadecadienoic (18:2Δ9*t*,12*c*)(and *trans*-9, *trans*-12-octadecadienoic (18:2Δ9*t*,12*t*) (16,23,24,26). It has been reported (26) that another major diethylenic *trans* isomer is present in the C_{18} region. Recently (16,23,24), this has been partially characterized as a *cis*,*trans* and/or *trans*, *cis* nonmethylene interrupted octadecadienoic acid. The exact position of the two double bonds and the respective geometries are yet to be determined. It should be cautioned that this nonmethylene interrupted diene (Peak No. 20 in Fig. 1) could be easily confused as 18:2Δ9*t*,12*t*, because these two isomers are almost eluted together in the GLC under normal operating conditions (16,24,26; also see Fig. 1). However, an improved resolution could be obtained using very efficient capillary columns (26) and by operating the GLC column at lower temperatures. *trans* Isomers of 18:3 are present in many dietary fats, but at trace or very low levels. Margarines made from canola and soybean oils contain higher levels of *trans* isomers of 18:3 than other vegetable oil margarines. Literature on this group of *trans* fatty acids is scarce, and their identity is not well established; in a previous report (16), we tentatively identified the principal isomers through GLC retention time data as *cis*-9, *cis*-12, *trans*-15; *trans*-9 *cis*-12, *cis*-15 and *cis*-9, *trans*-12, *cis*-15-octadecatrienoic acids [hereafter these isomers are collectively referred to as mono-*trans*-octadecatrienoic acid (18:3*t*)]. The ECL calculations on the SP-2340 FFSC indicated the probable presence of 18:3-di-*trans* isomers (16), but these are present at trace levels (<0.01%) and make no practical contribution to the total *trans*-content of the dietary fat.

Correction factors for relating the IR and GLC responses. As indicated above and elsewhere (1,2,5,16, 23,24,26), within each of the *trans* groups, namely *trans*-octadecenoate (18:1*t*),*cis*, *trans* or *trans*,*cis*-octadecadienoate [hereafter referred to as mono-*trans*-octadecadienoate (18:2*t*)], *trans*, *trans*-octadecadienoate (18:2*tt*) and mono-*trans*-octadecatrienoate (18:3*t*), various isomers are present, and in developing the mathematical formula it is assumed that there is no variation in the absorption between the various isomers within a *trans* group.

To determine the correction factor for the 18:1*t* group, several standard mixtures of methyl elaidate (18:1Δ9*t*) in methyl stearate (18:0) in varying proportions were prepared and analyzed by both GLC and IR. The percentage of 18:1*t* in the mixtures ranged from 5-35%, which covers the range for *trans*-monoethylenic fatty acids reported in most of the vegetable oil based margarines (1,2,4,5). Table 1 gives the actual weights, the GLC and the IR analytical results. The weight percent of 18:1Δ9*t* determined by GLC was in excellent agreement with the *trans* equivalent determined by IR with a correlation coefficient of 0.989; so that the correction factor for correlating IR and GLC responses for the 18:1*t* group is one.

The 18:2Δ9*t*,12*t* methyl ester was selected as the representative of the *trans*,*trans*-diene group because it is a major component in this group (5,16,23,24,26) and, further, it is the most readily available di-*trans* fatty acid. The IR and GLC results for mixtures of 18:0 and 18:2Δ9*t*,12*t* are given in Table 2A. The ratio of IR and GLC results for 18:2Δ9*t*,12*t* ranged from 1.68 to 1.80 with a mean value of 1.74. Therefore, the correction factor for correlating the IR and GLC responses for the 18:2*tt* group is 1.74. Generally, it has been assumed that the IR absorption caused by the two *trans* bonds in a di-*trans* fatty acid is twice that of methyl elaidate (23), but in Table 2A the data show that this assumption is not correct and the IR absorption for 18:2*tt* is only about 174% of that of methyl elaidate, which agrees closely with 166% as reported by Scholfield *et al.* (27) and 169% reported by Snyder and Scholfield (20).

TABLE 1

Comparison of GLC and IR Responses for 18:1Δ9*t* in a Mixture with 18:0

Actual amount (mg)		Wt% by GLC[a]		IR *trans*
18:0	18:1Δ9*t*	18:0	18:1Δ9*t*	unsaturation[a]
95	5	93.5	6.5	6.1
90	10	88.9	11.1	12.0
85	15	83.9	16.1	16.7
80	20	79.0	21.0	21.7
75	25	73.7	26.3	25.7
73	27.5	71.3	28.7	28.0
70	30	68.7	31.3	29.9
65	35	63.5	36.5	33.0

[a]Average of two analyses. GLC analyses were conducted on the SP-2340 column. Coefficient of correlation between the IR and GLC results for 18:1Δ9*t* is 0.989.

Authentic standards of mono-*trans*-octadecadienoates (18:2*t*) are neither commercially available nor readily accessible. However, a mixture containing 45.8% 18:2Δ9*c*,12*t* and 54.2% 18:2Δ9*t*,12*c* was synthesized in the laboratory. This 18:2*t* mixture was mixed with 18:0 and analyzed by both GLC and IR. Due to the limited availability of the synthetic 18:2*t*, only two sets of the 18:0/18:2*t* mixture could be prepared. The results in Table 2B show that the 18:2*t* has only about 84% of the IR absorption of methyl elaidate, which agrees with 85% reported by Scholfield *et al.* (27). Therefore, the correction factor relating the GLC and IR responses for 18:1*t* group is 0.84. The data presented in Table 2B demonstrate that response for the single *trans* bond in a *trans,cis*- or a *cis,trans*-diethylenic fatty acid is less than that of a *trans* bond in a monounsaturate.

The mono-*trans*-octadecatrienoates (18:3*t*) are also not readily available and are difficult to synthesize. The above results for 18:2*t* and 18:2*tt* support the observation of Scholfield *et al.* (27) that the intensity of the IR absorption of the *trans* bond in a polyunsaturated system is suppressed by the other *cis* and *trans* ethylenic bonds in the molecule. This means that the IR absorption of 18:3*t* has to be less than that of methyl elaidate. In the absence of any authentic standards, it is necessary and perhaps reasonable to suppose that the mono-*trans*-triene will absorb to the same extent as that of a mono-*trans*-diene, so that the correction factor for 18:3*t* is taken as 0.84. This correction factor for 18:3*t* may or may not be correct, but would be close to the actual value. In any case, considering the very low levels (0–0.8%) of 18:3 *trans* fatty acids present in hydrogenated vegetable oils (16; also see Table 3) the use of this correction factor of 0.84 would not impart any serious error in using the mathematical formula proposed below.

Mathematical formula: IR vs GLC. Using the correction factors, the total *trans* unsaturation of a margarine sample or any other dietary fat made from partially hydrogenated vegetable oil determined by IR, is correlated to the GLC weight percentages of the component *trans* fatty acids as given below:

IR *trans* = %18:1*t* + 0.84 × %18:2*t* + 1.74 × %18:2*tt* + 0.84 × %18:3*t*

The validity of this formula was tested on seven margarine samples of well established fatty acid composition. The detailed fatty acid compositions were evaluated as described previously (16) by the combined methoxy-bromo-mercuric adduct $AgNO_3$-TLC and GLC procedure (9). This method gave all the individual fatty acids in the sample, including all the *trans* groups and the total 18:1 *cis* and *trans*. The IR total *trans* unsaturation and the *trans* equivalents calculated from the GLC weight percentages by the above formula for the seven margarines samples are given in Table 4. There was an excellent agreement with a correlation coefficient of 0.989 for the total *trans* determined by the two methods.

As discussed above, due to the incomplete separation of *cis* and *trans*-monoenes direct GLC does not accurately determine the total 18:1*t* and the total 18:1*c*. The mathematical formula proposed here in conjunction with GLC and IR forms the basis for the determination of

TABLE 2

Comparison of GLC[a] and IR[a] Responses for 18:2Δ9*t*,12*t* and 18:2*t*[b] in Mixtures with 18:0

	Actual amount (mg)		Wt% by GLC		IR *trans*	Ratio of IR to GLC responses
A)	18:0	18:2*tt*	18:0	18:2*tt*		
	96	4	95.4	4.6	8.3	1.80
	92	8	90.9	9.1	15.8	1.74
	88	12	86.5	13.5	23.2	1.72
	80	20	77.3	22.7	38.1	1.68
						Mean ± S.D. = 1.74 ± 0.05
B)	18:0	18:2*t*	18:0	18:2*t*		
	95.8	4.4	95.3	4.7	4.0	0.85
	91.6	8.8	90.9	9.1	7.5	0.82
					Mean	= 0.84

[a]Average of two analyses. GLC analyses were conducted on the SP-2340 column.

[b]18:2*t* was a mixture of 45.8% 18:2Δ9*c*,12*t* and 54.2% 18:2Δ9*t*,12*c*.

TABLE 3

Fatty Acid Composition[a] of Some Canadian Margarines as Determined by the Combined GLC-IR Procedure

	Print margarine				Tub margarine				
Fat source	SO	VO/P	VO/L	CO	SO	VO/P	CO	OO	SF
Fatty acid	wt %								
16:0	23.9	22.0	8.9	16.0	24.9	10.2	16.2	14.2	5.9
18:0	6.7	7.5	8.7	5.4	4.9	5.3	5.6	7.4	10.4
Saturates[b]	31.3	31.5	17.6	21.7	30.3	15.8	21.8	22.4	16.3
18:1*t*[c]	30.2	31.6	37.2	27.1	11.6	18.9	13.5	19.3	12.6
18:1*c*[c]	21.4	27.9	35.0	21.7	19.0	43.4	23.1	47.2	20.5
Monoenes[b]	51.8	59.5	72.2	48.8	30.6	62.8	36.6	67.5	33.1
18:2Ω6	8.0	5.5	4.8	27.5	30.7	14.8	38.6	9.7	48.1
18:3Ω3	0.4	—	0.6	0.6	4.2	5.3	1.4	0.2	0.2
PUFA[d]	8.4	5.5	5.4	28.1	34.9	20.1	40.0	9.9	48.3
18:2*tt*	0.3	0.3	1.4	0.2	TR	0.2	TR	TR	0.4
18:2*t*	7.6	2.9	3.1	1.7	0.9	0.4	1.7	0.1	0.9
18:2*cc*[e]	0.3	0.5	TR	—	—	TR	—	—	0.7
18:3*t*	0.4	—	TR	—	—	0.8	—	—	0.3
trans(IR)	37.3	33.5	42.2	30.7	12.4	19.5	14.9	19.4	14.3
18:1*t*[f]	21.8	22.1	32.3	21.4	9.0	13.0	10.6	14.2	12.2
18:1*c*[f]	29.8	37.4	39.9	27.4	21.6	49.3	20.0	52.3	20.9
% Error[g]	27.9	30.1	13.2	21.0	22.4	31.2	21.5	26.4	3.2

[a]GLC analyses performed on the SP-2340 FFSC.

[b]Total includes fatty acids of other chain lengths.

[c]Determined by the combined GLC-IR procedure.

[d]Essential polyunsaturated fatty acids.

[e]Positional isomers of 18:2 other than 18:2Ω6.

[f]Determined by GLC alone; it is assumed that there is no overlap of *cis* and *trans* of 18:1. The first eluting 18:1 peak was considered as *trans* and all the other 18:1 peaks as *cis*.

[g]% Error of 18:1*t* value if determined by GLC alone. Fat source as given in the labels in the margarine samples: SO, soybean; VO/P, vegetable oil/palm; VO/L, Vegetable oil/lard; CO, corn; OO, olive; and SF, sunflower.

TABLE 4

Fatty Acid Composition and Comparison of the Total *trans* Equivalents of Margarines Calculated by the Formula with that Determined by IR

	Margarine (wt %)						
Fatty acids	1	2	3	4	5	6	7
Saturates	21.9	22.2	20.6	27.0	17.6	14.3	31.8
18:1*c*	22.7	27.6	28.8	29.7	34.5	50.3	18.3
18:1*t*	26.2	27.0	40.8	34.2	37.7	12.5	11.7
18:2*tt*	0.2	0.7	2.8	1.4	1.4	0.1	TR
18:2*t*	1.7	6.2	4.6	3.3	3.1	0.8	TR
18:2Ω6	27.8	14.6	1.3	3.5	4.8	14.9	37.5
18:3*t*	—	0.3	—	TR	TR	0.6	TR
18:3Ω3	0.6	1.3	0.2	0.9	0.6	6.4	0.7
Total *trans*/IR	28.2	32.4	50.2	38.6	42.1	14.9	11.1
Cal. *trans* equ.[a]	27.9	33.7	49.6	39.4	42.7	13.9	11.7

[a]The *trans* equivalents was calculated using the mathematical formula given in the text, which incorporates the GLC wt % of 18:1*t*, 18:2*tt*, 18:2*t* and 18:3*t* and their respective correction factors. GLC analyses were conducted on the SP-2340 column. The coefficient of correlation between the calculated and the IR results for the total *trans* equivalents is 0.998.

the total 18:1*t* and the total 18:1*c* in partially hydrogenated vegetable oils. As illustrated in Figure 1 and as demonstrated previously (16), capillary GLC on the cyanosilicone liquid phases resolve the 18:1, 18:2 and 18:3 fatty acid classes from each other with no serious overlaps. The various positional and geometrical isomers of 18:2 and 18:3, which are normally encountered in dietary fats, are also resolved with no significant interferences. Thus, GLC provides the proportions of the 18:2*t*, 18:2*tt* and the 18:3*t*, while IR yields the total *trans* unsaturation. Therefore, the total 18:1*t* can be calculated from the mathematical formula. The difference between the total 18:1 fatty acids which could easily be obtained by direct GLC analysis (Fig. 1) and the above calculated total 18:1*t* gives the total 18:1*c*. In most of the margarines and other dietary fats of vegetable oil origin, octadecenoic acid is the predominant, if not the sole, monoethylenic fatty acid. Monoethylenic fatty acids of C_{14}, C_{16}, C_{20} and C_{22} are invariably present, but at negligible concentrations. Thus the total 18:1*c* and total 18:1*t* could be considered approximately equal to the total *cis* and total *trans*-monoethylenic fatty acids, respectively. If appreciable levels of other monoethylenic fatty acids are present, then the mathematical formula has to be modified to incorporate them. An important criterion for application of this proposed combined GLC-IR method is that the sample should not contain measurable levels of conjugated *trans* fatty acids and nonfatty acid material containing *trans*-ethylenic unsaturation which may affect the IR absorption. It has been reported that conjugated *trans* fatty acids increase the absorption of the baseline of the analytical band at 10.3 μ and this results in errors if there is an appreciable amount (>5%) of these fatty acids present (28). Fortunately, most of the vegetable oil-based margarines contain very low levels of conjugated fatty acids (4,5), but values as high as 1.4% of the fatty acids have been reported for some margarines (8).

Table 3 gives the fatty acid analyses of nine brands of Canadian margarines as determined by the combined GLC-IR procedure. For comparison, the total 18:1*c* and 18:1*t* determined by GLC alone, the method that has been generally used by many workers, are given at the bottom of Table 3. For all the margarines, the total 18:1*t* were higher and the total 18:1*c* were lower when determined by the combined GLC-IR procedure as compared to the direct GLC method. These discrepancies are the consequence of disregarding the important overlaps of some of the 18:1*c* and 18:1*t* isomers on GLC by the direct GLC method. Table 3 clearly demonstrates that the direct GLC method underestimates the 18:1*t* values by substantial margin in favor of the *cis* isomers.

The GLC-IR procedure described here is simple, rapid and accurate, requires minimal time in sample preparation and involves just the direct analysis of the dietary fat as methyl esters by capillary GLC on a polar liquid phase and analysis of the same methyl ester sample by IR. This method does not require the fractionation of the fatty acid classes by $AgNO_3$-TLC or other means prior to GLC (8,9,16). The combined GLC-IR method should be useful for routine analysis of *cis* and *trans*-monoethylenic fatty acids in margarine and other dietary fats made from vegetable oils and animal fats. This procedure, as it is, cannot be applied to hydrogenated fish oils, because these fats contain a complex mixture of *cis-trans* isomers of highly polyunsaturated fatty acids, particularly of the C_{20} and C_{22} chain lengths (29). Their isomers are not easily resolvable by GLC, which severely impedes the development of a simple mathematical formula such as that proposed above for hydrogenated vegetable oils.

Recently Mossoba *et al.* (24) discussed a capillary gas chromatography/matrix isolation/Fourier Transform Infrared Spectroscopy (GC/MI/FT-IR) technique for the detection and quantitation of *trans* fatty acids in partially hydrogenated oils. It appears that in fats and oil laboratories where the GC/MI/FT-IR instrumentation is available, this technique could be adopted to measure directly the amount of 18:1*t* isomers coeluting with the *cis* isomers. With the GC/MI/FT-IR technique, the mathematical formula proposed here is not required as the amount of 18:1*t* could be measured directly. As mentioned in the introduction, it is not feasible to separate the 18:1 *cis* and *trans* isomers, even on very polar cyanosilicone flexible fused silica capillary columns. The major 18:1*c* peak contains important amounts of *trans* isomers (16,26). However, Mossoba *et al.* (24), using a cyanosilicone capillary column in the GC of GC/MI/FT-IR failed to detect any *trans* isomers in the major 18:1*c* peak. This probably shows that the sensitivity of the GC/MI/FT-IR technique is not adequate to measure the low levels of *trans* compounds when present as mixtures with major *cis* isomers. Perhaps for accurate measurements, as with any other technique, GC/MI/FT-IR may require extensive calibration with various proportions of *c* and *t*-18:1 isomers representative of those present in partially hydrogenated oils.

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