Analysis of $C_{18:1}$ *cis* and *trans* Fatty Acid Isomers by the Combination of Gas–Liquid Chromatography of 4,4-Dimethyloxazoline Derivatives and Methyl Esters

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ABSTRACT: *Trans* fatty acids in foods are usually analyzed by gas-liquid chromatography (GLC) of fatty acid methyl esters (FAME). However, this method may produce erroneously low values because of insufficient separation between *cis* and *trans* isomers. Separation can be optimized by preceding silver-ion thin-layer chromatography (Ag-TLC), but this is laborious. We have developed an efficient method for the separation of 18-carbon *trans* fatty acid isomers by combining GLC of FAME with GLC of fatty acid 4,4-dimethyloxazoline (DMOX) derivatives. We validated this method against conventional GLC of FAME, with and without preceding Ag-TLC. Fatty acid isomers were identified by comparison with standards, based on retention times and mass spectrometry. Analysis of DMOX derivatives allowed the 13*t,* 14*t,* and 15*t* isomers to be separated from the *cis* isomers. The combination of the GLC analyses of FAME and DMOX derivatives gave results comparable with those obtained by GLC of FAME after preceding Ag-TLC, while saving about 100 h of manpower per 25 samples. It allowed the identification and quantitation of 11 *trans* and 8 *cis* isomers and resulted in 25% higher values for total $C_{18:1}$ *trans,* compared with the analysis of FAME alone. The combination of DMOX and FAME analyses, as applied to the analysis of 14 foods that contained ruminant fat and partially hydrogenated vegetable and fish oils, indicated that the most common isomers were 11*t* in ruminant fats, 9*t* in partially hydrogenated fish fats, and either 9*t* or 10*t* in partially hydrogenated vegetable fats. The combination of GLC analyses of FAME and DMOX derivatives of fatty acids improves the quantitation of 18-carbon fatty acid isomers and may replace the laborious and time-consuming Ag-TLC.
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KEY WORDS: Diet, dimethyloxazoline derivatives, fatty acid isomers, foods, gas–liquid chromatography, methyl esters, *trans* fatty acids.

Isomeric *trans* fatty acids, found in partially hydrogenated vegetable and fish oils, dairy products, and meat from ruminant animals, have gained increasing interest because they adversely affect human serum lipoprotein composition (1), and their consumption may increase risk of coronary heart disease (2,3).

Gas–liquid chromatography (GLC) of fatty acid methyl esters (FAME) is the traditional method for the separation of fatty acids (4), and development of methods for long capillary columns with polar stationary phases has made it possible to separate most *trans* isomers from *cis*-unsaturated fatty acids (5). However, the separation between *trans* and *cis*-isomers is never complete in the GLC analysis of FAME, and particularly the 13*t* to 15*t* isomers of $C_{18:1}$ are overlapped by the 9*c*-isomer.

Improved separation of monoenoic *trans*- and *cis*-isomers is achieved by thin-layer chromatography on silver nitrate-impregnated silica plates (Ag-TLC) (6) or silver-ion liquid chromatography (Ag-HPLC) (7), followed by GLC (5,8,9), but this method is laborious. Improvements have been achieved by optimizing the temperature program (9). However, only complete separation of *cis* and *trans* isomers will provide accurate estimates of the *trans* fatty acid content of foods. Quantitation of vaccenic acid (11*t*), which is the main isomer of milk fat (8,10,11), and of elaidic acid (9*t*) and 10*t* octadecenoic acid, which represent the major components in most hydrogenated vegetable oils (8,12), would allow the identification of the source of *trans* fatty acids in processed foods and mixed diets. This identification is relevant because it has been suggested that *trans* fatty acids from dairy products and those from partially hydrogenated vegetable oils may have different effects on risk of coronary heart disease (2,13).

The ideal method for the analysis of 18-carbon *trans* fatty acids should combine complete separation from *cis*-isomers with good separation between different 18-carbon *trans* isomers. For these purposes, we have developed a method based on GLC of dimethyloxazoline (DMOX) derivatives of fatty acids (14), validated it with the Ag-TLC-GLC method for both FAME and DMOX derivatives of fatty acids, and applied it in combination with GLC of FAME to the analysis of selected foods with different fatty acid compositions.

MATERIALS AND METHODS

Food samples. For the validation study, 11 foods from different manufacturers, representing a broad range of *trans* fatty

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acid contents, were purchased locally in the Netherlands. These included four margarines/shortenings, four frying fats that contained hydrogenated vegetable oils, a composite sample of cake from nine different brands, one snack composite sample (eight pastries with sausage from different manufacturers) and one sample of butter. Foods were homogenized with a model B-400 mixer (Büchi Laboratoriumtechnik AG, Flawil, Switzerland) and stored at −20°C until analyzed.

For the applied study, samples were chosen from the foods collected for the European Multicentre TRANSFAIR Study in 1995–96 (15). They were selected from The Netherlands, Iceland, and Finland to represent fats of different ruminants and both hydrogenated vegetable oils and fish oils, and included three frying fats, butter, margarine, shortening, partially hydrogenated rapeseed and soybean oils, deep-fried foods (French fries, meat croquette), and meat (beef, lamb, reindeer, and elk). The samples had been homogenized and frozen in the respective countries, transported to The Netherlands stored frozen in tight plastic containers, and kept at −20°C until analyzed.

The Dutch samples for the validation study were analyzed by GLC of FAME and DMOX derivatives, both with and without preceding separation by Ag-TLC, with identification of fatty acid DMOX derivatives by mass spectrometry (MS). The multicenter TRANSFAIR samples were analyzed by GLC of FAME and DMOX derivatives without preceding Ag-TLC.

Preparation of FAME. Fats were extracted with chloroform/methanol (food samples) or hexane (edible fats), and FAME were prepared according to Metcalfe *et al.* (16).

Preparation of DMOX derivatives. To ~5 mg FAME, 500 µL 2-amino-2-methyl-1-propanol (AMP) was added and the mixture was incubated overnight at 180 °C. After cooling, 5 mL dichloromethane was added, and the total solution was washed twice with 2 mL demineralized water. The dichloromethane solution was dried with anhydrous sodium sulfate and subsequently evaporated under nitrogen. The residue was dissolved in 200 µL hexane (14).

Reference materials. Standard preparations of $C_{18:1}$ isomers were purchased from Sigma (St. Louis, MO) and Nu-Chek-Prep, Inc. (Elysian, MN), and included the 6, 7, 9, 11, 12, 13, and 15 *trans* isomers and the 6, 7, 9, 11, 12, 13, and 15 *cis* isomers.

GLC of FAME and DMOX. For the separation of $C_{18:1}$ fatty acid isomers, a Hewlett-Packard gas chromatograph (HP, Avondale, PA), was used, equipped with a 100-m capillary column (two 50-m columns coupled together, Chrompack, Middelburg, Netherlands). Details of the GLC methods are shown in Table 1. The results are expressed as area %.

Identification of fatty acid isomers by GLC and MS. The areas of fatty acid peaks of $C_{18:1}$ isomers between 5*c* and 15*c* and between 6*t* and 16*t* and of the *t/t, c/t, t/c,* and *c/c* isomers of $C_{18:2}$ were measured with HP Chem software. Individual peaks were identified by comparison with reference standards, based on retention times and MS analysis for the position of the double bonds, by using the DMOX derivatives. The MS analysis was performed as described by Fay and Richli (14). We used an HP 5890 Series II gas chromatograph, equipped with a 100 m × 0.25 mm CP-Sil 88 capillary column and an HP 5971 series mass spectrometer, El, 70 eV ionization mode and a mass range of 50–400 atomic mass units (AMU). Split injection (split ratio 1:50) and temperature programming from 150 to 220°C were used with helium as carrier gas at a flow of 23.8 cm/s (0.7 mL/min). The position of the double bond was determined by an interruption of the regular pattern of successive chain cleavages of methylene units of 14 AMU and the observation of an interval of 12 AMU (17,18) (Fig 1).

Ag-TLC. TLC plates (20 × 20 cm, Art. 11798; Merck, Darmstadt, Germany) were impregnated in a TLC-tank that contained about 15% $AgNO_3$ in acetonitrile. The $AgNO_3$ solution was allowed to ascend until the solvent front had reached the top of the plate (19). Shortly before use, the plates were activated by drying them at 110°C for 1 h (6).

In the concentration zone of the plate, 200 µL FAME (~6 mg) was applied as a narrow linear band. We used petroleum ether/diethyl ether (95:5 vol/vol) as mobile phase. After dry-

TABLE 1
Details of the Gas–Liquid Chromatographic Methods

	$C_{18:1}$ FAME	$C_{18:1}$ DMOX
GLC equipment	HP 5890 II+	HP 5890 II+
Column length/internal diameter film thickness	CP-Sil 88 capillary 100 m/0.25 mm 0.20 µm	CP-Sil 88 capillary 100 m/0.25 mm 0.20 µm
Injection/split ratio	split/1:88	split/1:75
Carrier gas linear velocity (flow rate)	H_2 24.8 cm s^{-1} (1 mL min^{-1})	H_2 19.6 cm s^{-1} (0.7 mL min^{-1})
Temperature program	From 150 to 155°C (2°C/min), then 50 min constant temperature, program to 170°C (40°C/min), then 7.12 min constant temperature, program to 224°C (40°C/min) and 8.66 min constant temperature.	From 150°C (for 125 min) to 220°C (10°C min^{-1}) and constant temperature for 13 min.
Total run time	70 min	145 min

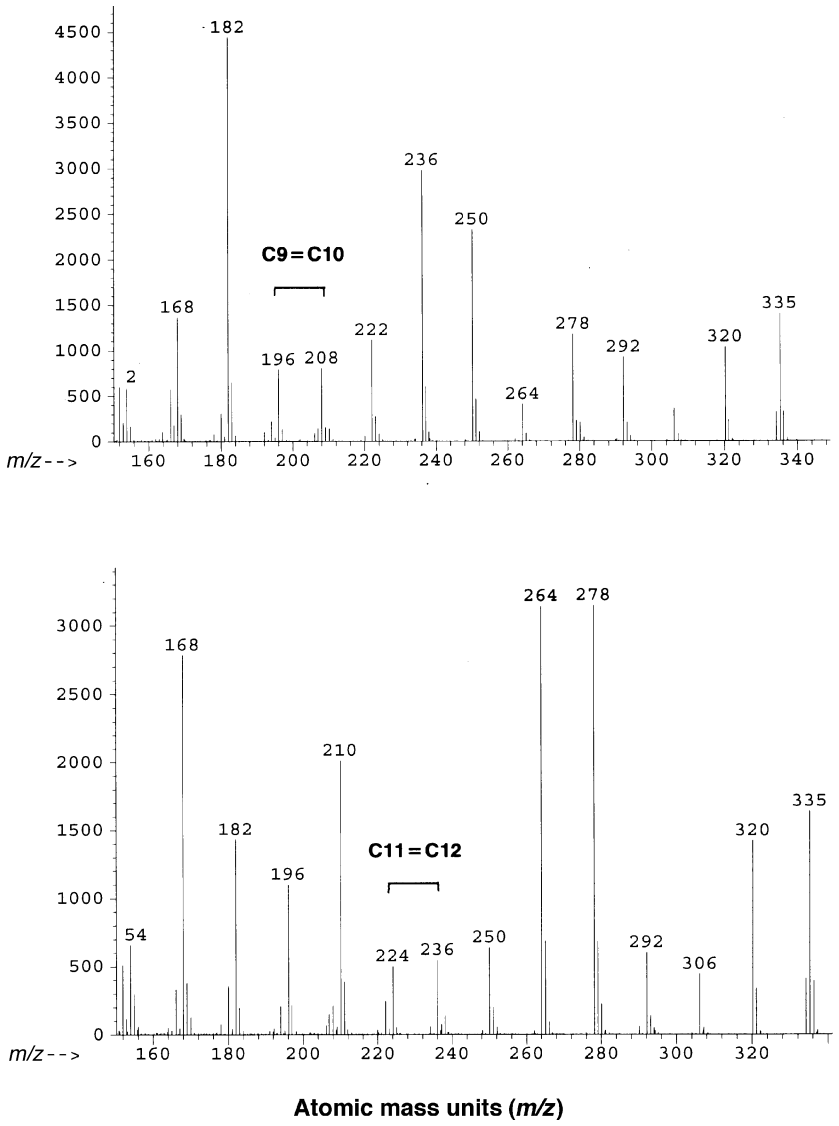

FIG. 1. Mass spectra of the DMOX derivatives of 9-*trans* $C_{18:1}$ showing a double bond between atomic mass units 196 and 208 (upper panel), and 11-*trans* $C_{18:1}$ with a double bond between atomic mass units 224 and 236 (lower panel).

ing, the plate was sprayed with a solution of Rhodamine 6G (25 mg/100 mL ethanol), and the bands with saturated, *trans*-monounsaturated, and *cis*-monounsaturated fatty acids were visualized under UV radiation. The three bands, and a fourth band covering the area between the *trans*- and *cis*-bands, were scraped off separately, transferred to filtration tubes, and washed three times with 10 mL ethyl ether. The eluents were collected in receivers that contained a known amount of $C_{17:0}$ FAME as internal standard. The eluents were dried in a rotator evaporator, dissolved in 750 µL petroleum ether, and analyzed by GLC.

RESULTS

Validation study. Recovery of fatty acids after Ag-TLC. When the fatty acids of the three TLC-bands of saturated, *cis*-unsaturated, and *trans*-unsaturated fatty acids were added together, the recovery of total fatty acids was only ca. 85%, compared with the GLC of FAME without Ag-TLC. After inclusion of the fourth (intermediate) band, which contained both *cis*-isomers (mainly 11*c*–13*c*) and *trans*-isomers (mainly 6*t*), the recovery was increased to 95.6% (range 86.7–103.8%).

Analysis of FAME. In the GLC analysis of FAME, seven *trans* and six *cis* isomers of $C_{18:1}$ were identified, although with overlap between isomers. The 13*t*, 14*t,* and 15*t* isomers were overlapped by the 9*c* and 11*c* isomers, 7*t* and 8*t* could not be separated from each other, and 12*t* could not be separated from 5*c* and 7*c* (Fig. 2, Table 3). Preceding Ag-TLC improved the separation and allowed individual calculations of the proportions of 12*t,* 13*t* + 14*t*, 15*t,* and 5*c* + 7*c* isomers. This increased the number of identified fatty acids to nine *trans* and seven *cis* isomers (Table 2). Otherwise, the analy-

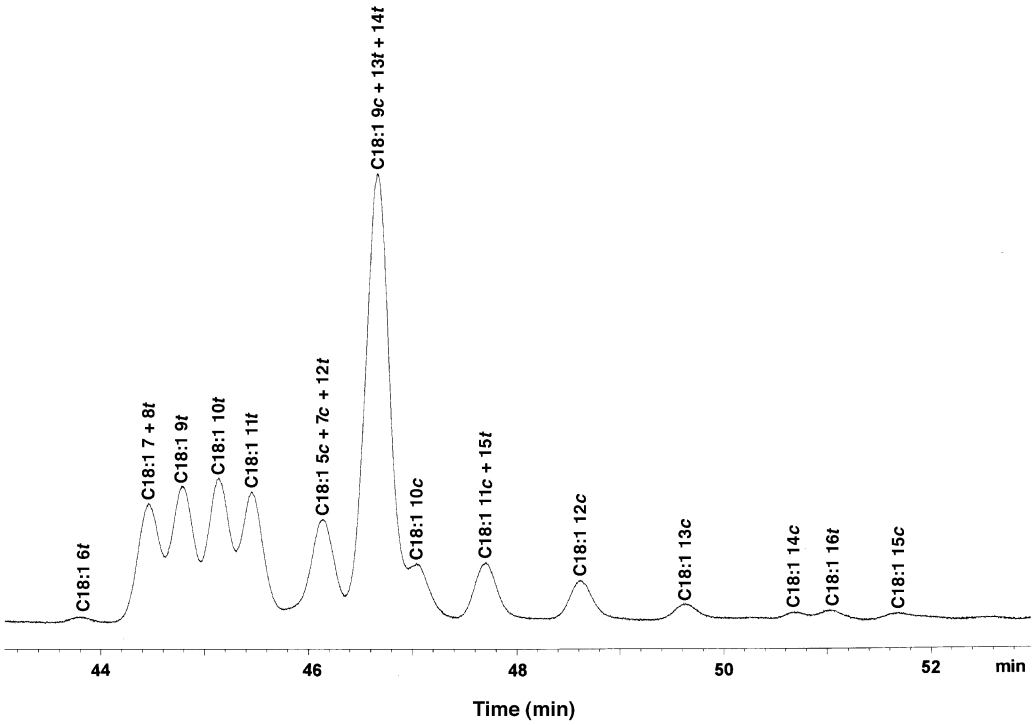

FIG. 2. Capillary gas chromatogram of the $C_{18:1}$ region of a food sample (French fried potatoes) as FAME on a CP-Sil 88 column.

TABLE 2
The 18-Carbon Fatty Acid Composition of 11 Dutch Foods, Analyzed as % of Methyl Esters (FAME) and Dimethyloxazoline Derivatives (DMOX), With and Without Preceding Ag-TLC, and by the Combination of FAME and DMOX Without Preceding Ag-TLC. Values are Means (and Ranges)

	FAME		DMOX		FAME
	Without	With	Without	With	+
$C_{18:1}$	Ag-TLC	Ag-TLC	Ag-TLC	Ag-TLC	DMOX
6*t*	0.1	0.1	0.1	0.2	0.1
7*t*	[a]	[a]	[a]	[a]	0.8
8*t*	3.0	2.9	2.9	2.5	2.7
9*t*	4.0	4.0	4.8	4.3	4.0
10*t*	3.6	3.6	4.1	3.7	3.6
11*t*	2.8	2.9	2.9	3.1	2.8
12*t*	2.7	1.7	1.8	1.6	1.8
13*t*	[a]	[a]	1.0	0.9	1.0
14*t*	[a]	1.6	0.5	0.4	0.5
15*t*	[a]	0.3	0.2	0.1	0.2
16*t*	0.3	0.2	[a]	[a]	0.3
5*c*	[a]	[a]	[a]	0.4	0.7
7*c*	2.7	1.3	[a]	0.8	0.5
9*c*	22.4	21.2	21.4	22.0	21.4
10*c*	1.1	1.1	1.0	1.1	1.1
11*c*	1.9	1.5	1.6	1.5	1.6
12*c*	1.2	1.2	1.1	1.0	1.2
13*c*	0.4	0.3	0.3	0.2	0.4
15*c*	0.2	0.2	0.3	0.2	0.2
Total $C_{18:1}t$	13.8 (0.3–42.4)	17.3 (0.3–51.0)	18.2 (0.0–54.6)	17.2 (0.0–51.1)	17.2 (0.3–51.0)
Total $C_{18:2}t$	1.1 (0.0–2.7)	1.0 (0.0–2.3)	0.4 (0.1–0.9)	0.6 (0.1–1.2)	1.1 (0.0–2.7)
Total *trans*	15.0 (0.3–45.1)	18.4 (0.3–53.3)	18.7 (0.1–52.0)	17.9 (0.1–52.0)	18.4 (0.3–53.7)
Total $C_{18:1}c$	29.8 (19.7–36.3)	26.7 (19.7–34.0)	25.7 (16.5–32.6)	27.2 (19.3–35.2)	27.1 (18.9–33.8)

[a]Not separately detectable.

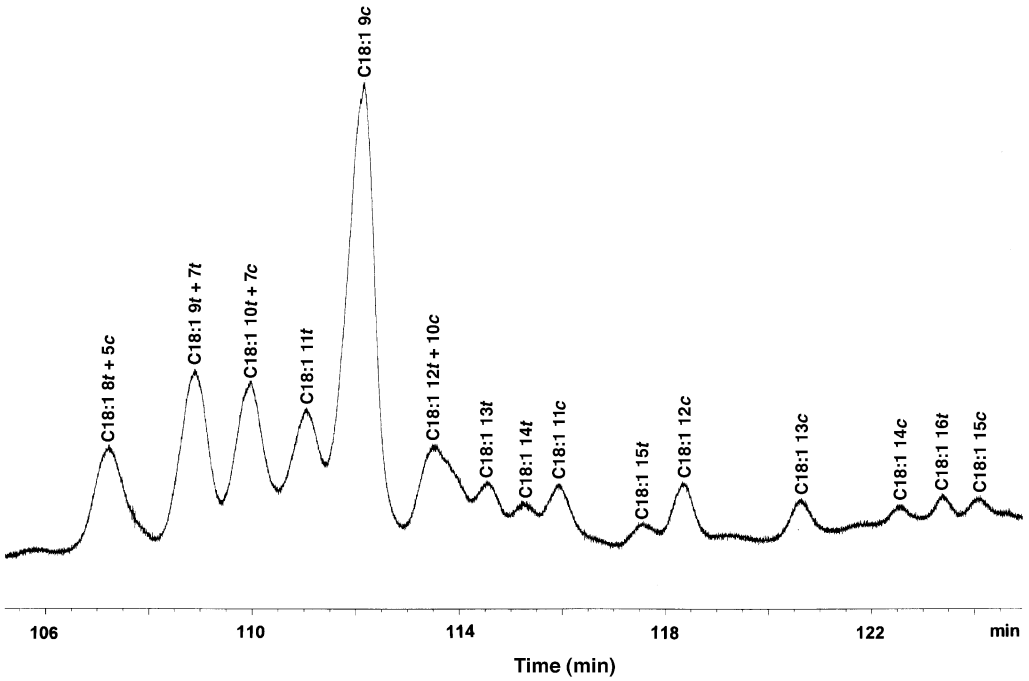

FIG. 3. Capillary gas chromatogram of the $C_{18:1}$ region of a food sample (French fried potatoes) as DMOX derivatives on a CP-Sil 88 column.

ses of FAME with and without Ag-TLC gave rather similar proportions of individual fatty acid isomers, but the *trans* fatty acid contents were underestimated by FAME without Ag-TLC because of overlapping of the 12*t*–15*t* isomers by the 7*c*, 9*c* and 11*c* isomers. Small amounts (< 0.3%) of $C_{16:1}$ *trans* fatty acids were found in three samples only. $C_{18:2}$ *trans* isomers comprised on average 1.1% (range 0–2.7%) of total fatty acids.

Analysis of DMOX derivatives. The GLC analysis of DMOX derivatives yielded nine *trans* and six *cis* isomers of $C_{18:1}$, but with some overlap between the 7–9*t* and the 5*c* and 7*c* isomers (Fig. 3, Table 2). The separation of the peaks differed from the analysis of FAME in several respects. The 13*t*, 14*t,* and 15*t* isomers were separated from 9*c,* but 16*t* was poorly detectable. The 7*t* isomer could not be separated from the 9*t* isomer. The 5*c* isomer overlapped with 8*t,* and the 7*c* isomer with 10*t.* These isomers could be separated by preceding Ag-TLC (Table 2).

Combination of FAME and DMOX analyses without Ag-TLC. The proportions of the 6*t*, 9–11*t,* and 16*t* isomers, 10*c,* and 12–15*c* were derived directly from the GLC analysis of FAME (Table 3). The 12–15*t* and 9*c* isomers were derived from the separate GLC analysis of DMOX derivatives. The 7*t*, 8*t*, 5*c,* and 7*c* isomers were calculated by combining the FAME and DMOX chromatograms as follows. 7*t* = DMOX (7 + 9*t*) − FAME 9*t*; 8*t* = FAME (7 + 8*t*) − 7*t* (as calculated above); 5*c* = DMOX (8*t* + 5*c*) − 8*t* (as calculated above); 7*c* = DMOX (10*t* + 7*c*) − FAME 10*t*. In some samples of the applied study, the DMOX derivatives of 12*t* and 10*c* were poorly separated; in those situations, 12*t* was calculated by the difference between DMOX (12*t* + 10*c*) and FAME 10*c*. By these additional calculations, the number of identified and measured $C_{18:1}$ fatty acid isomers was increased to eleven *trans* isomers (6*t*–16*t*) and eight *cis* isomers (5*c*, 7*c,* and 9–15*c*) (Tables 2,3).

FAME and DMOX in comparison with FAME after Ag-TLC. The analysis of FAME gave consistently lower values for total $C_{18:1}$ *trans* fatty acids than FAME after preceding Ag-TLC (Table 2, Fig. 4). The combination of FAME and DMOX analyses gave values similar to those derived from FAME with preceding Ag-TLC, both for individual fatty acid isomers and for total $C_{18:1}$ *trans* fatty acids (Table 2, Fig. 5). The proportions of unidentified fatty acids were low, between 1.0 and 2.2% by all methods.

The time needed by technicians for the preparation and analysis of the samples (excluding the time of the GLC runs, which do not require human attendance) was calculated for a series of 25 samples for each method. Fat extraction required 30 h in each case, the additional time required in preparation for the conventional GLC of FAME was 13 h, that for the combination of FAME and DMOX analyses was 25 h, whereas the combination of Ag-TLC and FAME required 140 additional hours of manpower.

Applied study. The $C_{18:1}$ *trans* fatty acid compositions of the partially hydrogenated fish oils and vegetable oils were rather similar (Fig. 6), with the 9*t* isomer (elaidic acid) showing the highest proportions, between 21 and 41% of $C_{18:1}t$, in six out of the nine food products analyzed. In four foods, the lightly hydrogenated soybean oil from Iceland (Fig. 7) and the shortening, margarine, and French fries from The Netherlands, the 10*t* isomer was the most prevalent (23–26% of

TABLE 3
Separation of $C_{18:1}$ Fatty Acid Isomers by FAME and DMOX, and Calculation of the *cis* and *trans* Isomers by the Combination of FAME and DMOX Methods Without Preceding Ag-TLC

$C_{18:1}$ isomer	FAME	DMOX	Calculation by FAME and DMOX
6*t*	6*t*	6*t*	FAME
7*t*	7*t* + 8*t*	7*t* + 9*t*	DMOX (7*t* + 9*t*) – FAME 9*t*
8*t*	7*t* + 8*t*	8*t* +5*c*	FAME (7*t* + 8*t*) – DMOX (7*t* + 9*t*) + FAME 9*t*
9*t*	9*t*	7*t* + 9*t*	FAME
10*t*	10*t*	10*t* + 7*c*	FAME
11*t*	11*t*	11*t*	FAME
12*t*	12*t* + 5*c* + 7*c*	12*t* (+ 10*c*)[a]	DMOX or DMOX (12*t* + 10*c*) – FAME 10*c*
13*t*	13*t* + 14*t* + 9*c*	13*t*	DMOX
14*t*	13*t* + 14*t* + 9*c*	14*t*	DMOX
15*t*	15*t* + 11*c*	15*t*	DMOX
16*t*	16*t*	(16*t*)[b]	FAME
5*c*	5*c* + 7*c* + 12*t*	5*c* + 8*t*	DMOX (5*c* + 8*t*) – FAME (7*t* + 8*t*) + DMOX (7*t* + 9*t*) – FAME 9*t*
7*c*	5*c* + 7*c* + 12*t*	7*c* + 10*t*	DMOX (10*t* + 7*c*) – FAME 10*t*)
9*c*	9*c* + 13*t* + 14*t*	9*c*	DMOX
10*c*	10*c*	10*c* (+ 12*t*)[a]	FAME
11*c*	11*c* + 15*t*	11*c*	DMOX
12*c*	12*c*	12*c*	FAME
13*c*	13*c*	13*c*	FAME
15*c*	15*c*	15*c*	FAME

[a]12*t* and 10*c* are not always well separated; [b] often poorly detectable.

$C_{18:1}t$). *Trans* vaccenic acid (11*t*) comprised 14–22% of the $C_{18:1t}$ isomers in the partially hydrogenated vegetable fats. The $C_{18:2}$ *trans* isomers were low (0.2–0.3% of total fatty acids) in the fish oil-based fats and more variable in the vegetable oil-based products, ranging from 0.1% in deep-fried meat croquette to 2.5% in partially hydrogenated rapeseed oil.

The 11*t* (*trans* vaccenic acid) was the most common *trans* isomer in the fats of ruminants, comprising 46% of $C_{18:1t}$ in butter (Fig. 7) and 58 and 54% in beef and lamb meat, respectively. The proportion of the 11*t* isomer in reindeer and elk meat was somewhat lower, at 34% (Fig. 8). Correspondingly, 9*t* comprised only 6–7% of $C_{18:1t}$ in butter and beef and lamb meat, whereas it was more common (12–14% of $C_{18:1t}$) in the meat of reindeer and elk.

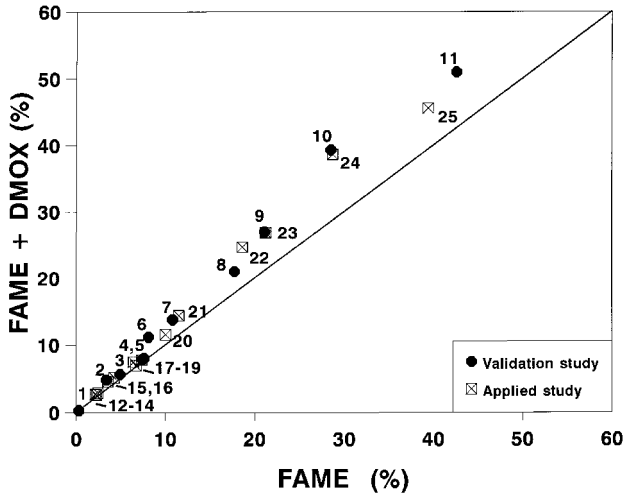

FIG. 4. Relation between total $C_{18:1}$ *trans* fatty acids as determined by the GLC analysis of FAME and by the combination of FAME and DMOX analyses in the 11 foods of the validation study and the 14 foods of the applied study. Validation study: 1, stick margarine; 2, butter; 3, semi-solid frying fat (wholesale); 4, sausage roll; 5, tub margarine; 6, frying fat (retail); 7, cake; 8, margarine for bakeries (wholesale); 9, frying fat (retail); 10, shortening (retail); 11, frying fat (retail). Applied study: 12, partially hydrogenated soybean oil; 13, reindeer meat; 14, elk meat; 15, salted butter; 16, beef; 17, fat for bakeries containing hydrogenated fish oil; 18, lamb meat; 19, frying fat containing hydrogenated fish oil; 20, partially hydrogenated rapeseed oil; 21, vegetable margarine; 22, meat croquette; 23, French fried potatoes; 24, frying fat containing hydrogenated soybean oil; 25, hard frying fat.

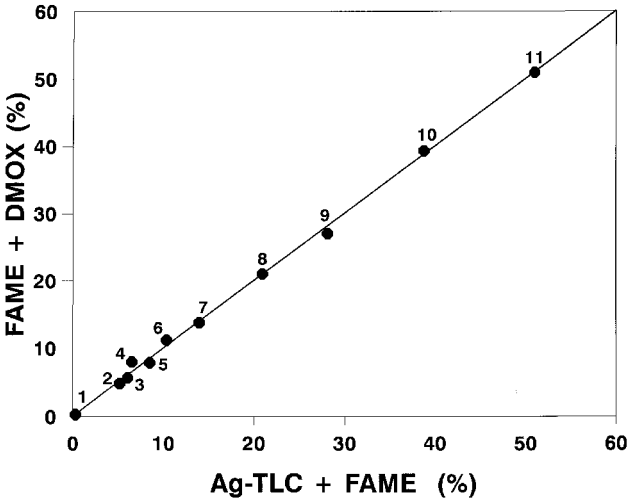

FIG. 5. Relation between total $C_{18:1}$ *trans* fatty acids as determined by the GLC analysis of FAME after Ag-TLC and by the combination of FAME and DMOX analyses in the 11 foods of the validation study. See Figure 4 for food numbers.

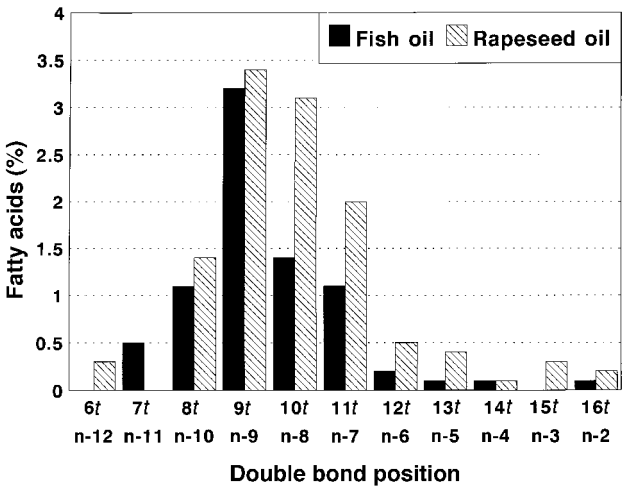

FIG. 6. The distribution of $C_{18:1}$ *trans* isomers in fats from Iceland containing partially hydrogenated fish oil and partially hydrogenated rapeseed oil.

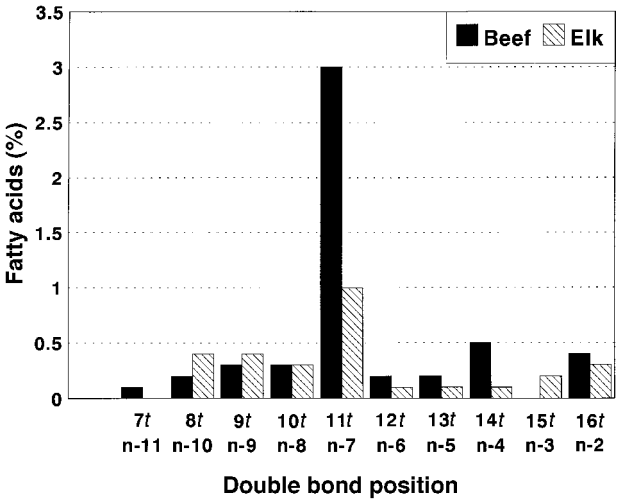

FIG. 8. The distribution of $C_{18:1}$ *trans* fatty acids in Dutch beef and Finnish elk meat samples.

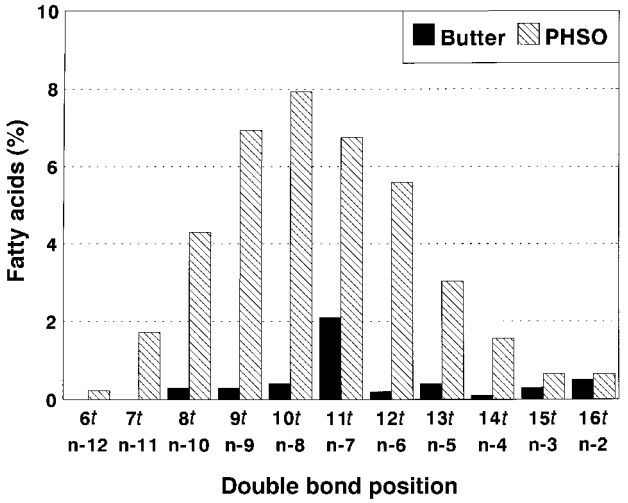

FIG. 7. The distribution of $C_{18:1}$ *trans* fatty acids in Dutch samples of butter and of shortening containing partially hydrogenated soybean oil (PHSO).

$C_{18:2}$ *trans* isomers were highest in butter, lower in beef and lamb meat, and lowest in reindeer and elk meat. Linoleic acid was low in butter and the meat of domestic ruminants, but its proportions were considerably higher in the meat of the wild animals, particularly in elk meat (15.6% of total fatty acids). Elk meat also contained considerable amounts (15.9%) of unidentified fatty acids.

DISCUSSION

Our results indicate that combination of the GLC analyses of FAME and DMOX derivatives is equivalent to the more complicated method of analyzing FAME after preceding Ag-TLC for the determination of total $C_{18:1}$ *trans* fatty acids in foods, and it exceeds the accuracy of the latter method in separating the *cis* and *trans* isomers of $C_{18:1}$. The fatty acid DMOX derivatives, described by Zhang *et al.* (20), possess good GLC characteristics due to their high volatility, and their mass spectra show easily recognizable peaks for the determination of the positions of double bonds (14,20). However, determination of the full fatty acid spectrum by GLC analysis of DMOX derivatives is slow, and the separation of small fatty acid peaks is often poor with this method. Therefore, it is more efficient to combine the GLC analyses of FAME and DMOX derivatives and use the latter only for identification and quantitation of those $C_{18:1}$ fatty acid isomers that are not adequately separated by the analysis of FAME. Another advantage of this combination is that DMOX derivatives can be prepared from FAME (14). By using the combination of GLC analyses, 11 different 18-carbon monoenoic *trans* fatty acid isomers could be quantified, together with eight *cis* isomers. Our results with the GLC of FAME with preceding Ag-TLC are in agreement with those of a previous study by Molkentin and Precht (8).

AOCS Method Ce 1c-89, based on GLC of FAME, often underestimates *trans* fatty acid levels (21). Attempts to overcome this shortcoming have included determination of total *trans* fatty acids by infrared spectroscopy (IR) in addition to GLC analysis (22). However, the precision of the GLC-IR method is low, and it is only applicable to samples with more than 5% *trans* fatty acids. By correct choice of the capillary column and exact adjustment of the temperature, it has been possible to improve the accuracy of the GLC analysis of FAME (9) and, for foods with relatively constant fatty acid compositions, such as dairy products, correction factors have been calculated for the estimation of total *trans* fatty acid intakes (8). However, the combination of FAME and DMOX analyses presented here allows accurate quantitation of all *cis* and *trans* isomers of $C_{18:1}$ fatty acids in foods of different origins and with variable fatty acid compositions.

By the use of Ag-TLC, it is possible to separate monoenoic

cis and *trans* isomers prior to GLC analysis. Two major problems of this method are quantitation and labor. The TLC bands are not quite straight and sharply separated from each other. In our study, 15% of total *trans* fatty acids would have been lost from the GLC analysis if only the visible fatty acid bands had been collected and the area between *trans* and *cis* monounsaturates had been left out. The loss of fatty acids is not random, but affects mainly certain isomers, such as 6*t* and 11–13*c* (6). Another drawback of the Ag-TLC method is the amount of time-consuming manual labor that is required. Our results show that replacing this laborious method with the combination of the GLC analyses of FAME and DMOX derivatives saves about 115 hours of technician's time per 25 samples.

The application of the combined FAME + DMOX method to the analysis of food samples selected from the European TRANSFAIR study revealed some differences between the isomeric fatty acid compositions of these foods. In agreement with previous studies, the hydrogenated vegetable oils contained a variety of $C_{18:1}$ *trans* isomers, with the 9*t* and 10*t* isomers being most prominent (12,23). The 10*t* isomer was the most common in products based on partially hydrogenated soybean oil, whereas 9*t* was higher in partially hydrogenated rapeseed oil (Figs. 6,7). Highest proportions of elaidic acid (9*t*) were found in partially hydrogenated fish oils from Iceland. Vaccenic acid (11*t*) was the most common isomer in ruminant fats, in agreement with previous studies (10,23), but its proportions were lower in reindeer and elk meat, compared with beef and lamb meat (Fig. 8). It is important to realize that many fats that were composed of hydrogenated vegetable oils contained considerably higher amounts of vaccenic acid than dairy products and ruminant meat (Fig. 7).

In conclusion, the conventional GLC analysis of FAME underestimates the amount of $C_{18:1}$ *trans* fatty acids by some 25% because of overlapping with *cis* isomers. Whenever complete separation of isomers and exact measurement of total fatty acid classes is necessary, the additional GLC analysis of DMOX derivatives and calculation of isomers by combining the results of FAME and DMOX analyses is recommended. This method can be used to replace the more laborious alternative of preceding silver-ion chromatography.

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