The Application of Packed Column Gas Chromatographic Analysis to the Determination of *trans* Unsaturation

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ABSTRACT AND SUMMARY

A study of the separation of synthetic mixtures of *cis* and *trans* methyl octadecenoate with packed column gas chromatography indicated its usefulness for such analyses. Several margarines were analyzed by this method and the results compared with those obtained by the infrared spectroscopic method. Reasonable agreement between both methods was obtained, but the gas chromatographic determination yielded lower values (average 3.8%). The presence of *trans* unsaturation was also shown in selected samples of human milk and blood lipids.

INTRODUCTION

The analysis of *trans* fatty acids has assumed renewed importance in the monitoring of fats produced via hydrogenation, as well as in the determination of the *trans* content of biological tissues and fluids resulting from dietary studies. Traditionally, the determination of *trans* acids has been carried out by infrared spectrophotometry (1). Infrared spectrophotometry is useful when the *trans* content is at reasonable levels and sufficient amounts of sample are available. Gas liquid chromatography (GLC) has the potential of analyzing both small samples and very low *trans* contents. However, such analyses have not been routinely carried out since the current technology for GLC analysis of the geometrical isomers depends primarily upon open tubular methodology using long capillary columns. This area has recently been reviewed (2). In addition, a more recent paper has appeared which indicated the development of an automated glass capillary gas chromatograph to the analysis of *cis/trans* isomers in plasma phospholipids (3). The successful analysis of lipids via capillary gas chromatography requires considerably more expertise, is more expensive, and consumes more time. Furthermore, capillary columns require more care to preserve their relatively short lives.

It would seem that a packed column method would be more acceptable to lipid laboratories to carry out analyses for *trans* acids on a routine basis. Very polar stationary phases are now available which make this feasible. Such phases are composed of cyanosilicone polymers coated on inactivated supports. Earlier work with Silar-10C (4) and SP-2340 (5) stationary phases indicated that some overlap in *trans/cis* separations occurred when low amounts of *trans* isomers were present with large amounts of the *cis* isomers. More recently, Ottenstein et al. (6) have shown that baseline separation can be obtained with columns packed with OV-275 stationary phase. Preliminary separations of the methyl esters of several margarines have been published (7). However, any method suggested for use with packed columns should be applicable to wide ranging concentrations of *trans* unsaturation. The results obtained should also be in agreement with those obtainable with the American Oil Chemists' Society official infrared method (1).

In the present work, we present results obtained for the analysis of several margarine samples and comparisons with those results obtained with infrared spectroscopy. The *trans* isomer composition of lipids from human milk, blood serum, and red blood cells was also determined.

EXPERIMENTAL PROCEDURES

Preparation of Margarine Methyl Esters

One pound each of twelve margarines purchased locally was melted with gentle heating and an aliquot of oil removed. The margarine oil was then filtered to remove suspended solids. Methyl esters of the oil were prepared by heating a mixture of ca. 1 g of the oil, 20 ml benzene, and 100 ml of 3% (v/v) H_2SO_4 in anhydrous methanol for 12 hr under reflux conditions to effect transesterification. The methyl esters so prepared were isolated by the addition of water, followed by extraction with petroleum ether. The organic extract was washed with water, dried over anhydrous sodium sulfate, and the solvent removed under vacuum with a rotary evaporator. The isolated methyl esters were stored at -5 C under a nitrogen atmosphere for a short time until utilized.

Human Milk Lipids

The total lipids from human milk (20-25 ml) were isolated by extraction with chloroform-methanol (2:1) (8).

Blood and Red Blood Cell Lipids

Freshly drawn, heparinized blood (5 ml) was centrifuged in a clinical centrifuge to remove red blood cells. The serum was retained for lipid extraction as were the washed red blood cells. Both serum and cells were extracted with chloroform-methanol (8) and the lipid recovered by gentle evaporation in vacuo with a vacuum evaporator.

Preparation of Methyl Esters

Methyl esters of serum and red blood cell lipids were separately prepared by solubilization of the lipid in a small amount of benzene and the addition of 20-25 ml of 3% (v/v) H_2SO_4 in anhydrous methanol, followed by heating under reflux for 12 hr. The methyl esters so formed were isolated as described in the preparation of margarine esters.

Standards

Standards (Nu Chek Prep, Elysian, MN and Supelco, Inc., Bellefonte, PA) of varying concentrations of methyl oleate and methyl elaidate were prepared both by direct weighing of the two standards and volumetric dilutions of standard solutions. Standard mixtures of fatty acid methyl esters were also employed to assist in identification of components eluted from the gas chromatograph.

Infrared Spectrophotometry

Provided that sample size was sufficient, 5% (w/v) solutions of methyl esters were prepared in carbon disulfide and the *trans* content determined according to the "Official and Tentative Methods of the American Oil Chemists' Society" (1). A Beckman IR-7 Infrared Spectrophotometer was employed.

Gas Chromatography

Both fatty acid and *trans* acid compositions of the prepared methyl esters were determined simultaneously with a

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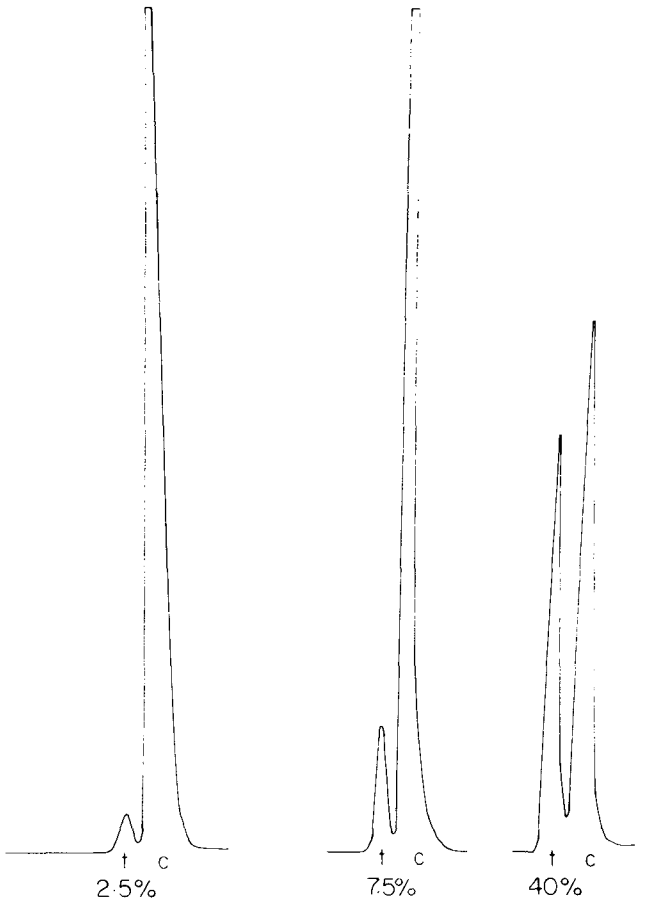

FIG. 1. Separation of 2.5, 7.5, and 40% methyl elaidate in the presence of methyl oleate: column conditions: 20 ft x 1/8 in. SS, packed with 15% OV-275 coated on 100/120 mesh Chromasorb P, AW-DMCS (7). Column temperature: 220 C; injector, 240 C; detector, 300 C; carrier gas N_2 at 9 ml/min. chart speed 0.3 cm/min. CR_t 18:1 tmm = 27.28 min).

Hewlett-Packard Model 5830 Flame Ionization Gas Chromatograph. The conditions employed were: column, 20 ft x 1/8 in. stainless steel packed with 15% OV-275 coated on 100/120 mesh Chromosorb P, AW-DMCS (7) (Supelco, Inc.); column temperature, 220 C; injector, 240 C; detector, 300 C; carrier gas, N_2, flow rate, 8-10 ml/min.

TABLE I

Analysis of Standard Mixtures of Methyl Oleate/Elaidate for *trans* Content via Packed Column Gas Liquid Chromatography[a]

Sample	% *trans*		Error	
	Actual[b]	Found[c]	Absolute	Percent Dev.
1	54.3	52.9	−1.4	2.5
2	50.0	50.9	+0.9	1.9
3	45.6	46.5	+0.1	2.1
4	43.1	41.2	−1.9	4.5
5	40.0	42.1	+2.1	5.3
6	30.0	31.4	+1.4	4.6
7	28.4	27.6	−0.8	2.8
8	27.8	27.3	−0.5	2.0
9	23.9	24.1	+0.2	1.1
10	20.0	20.9	+0.9	4.8
11	9.4	10.0	+0.6	6.4
12	7.9	8.5	+0.6	6.7
13	7.5	8.3	+0.8	10.6
14	5.0	5.2	+0.2	4.0
15	1.0	0.9	−0.1	10.0
			M 0.83 ± 0.62	M 4.62 ± 2.85

[a]20 ft x 1/8 in. SS column packed with 15% OV-275 coated on 100-120 mesh Chromosorb P (AW-DMCS). Column, 220 C; injector, 240 C; detector, 300 C; carrier gas flow, 10 ml/min.

[b]Prepared from pure standards by weighing with a semi-microbalance.

[c]Determined by area response via electronic integration on an HP 5730 gas chromatograph.

Approximately 10 μg of methyl esters were injected onto the column in 1 μl of isooctane as solvent. Quantitation was achieved by means of the automatic electronic integrator of the Hewlett-Packard 5830; column performance and accuracy of quantification was monitored by periodic analysis of standard mixtures of fatty acid methyl esters.

RESULTS AND DISCUSSION

Although still preliminary, the results obtained in this short study indicate that wide ranges of concentration of *trans* acids can be determined by packed column gas chromatography. The chromatogram illustrated in Figure 1 demonstrated that concentrations of elaidate as low as 2.5% are easily determined. Subsequent work has allowed the determination of ca. 0.2% *trans* when the amount of sample injected into the gas chromatograph is increased. The reasonably accurate determination of large ranges of *trans* isomers is a desirable goal for such a packed column

TABLE II

Fatty Acid Composition of Margarine Methyl Esters

Fatty acid	Wt % Margarine											
	A	B	C	D	E	F	G	H	I	J	K	L
12:0	-	-	-	-	-	-	0.1	0.1	0.2	-	-	-
14:0	0.1	-	-	0.1	0.1	0.1	0.3	0.3	0.1	0.1	0.1	-
16:0	11.4	11.7	11.6	10.5	11.3	11.5	16.9	16.6	11.9	10.8	11.5	11.3
16:1	0.1	0.1	-	0.2	0.2	0.2	0.1	0.2	0.2	0.2	0.2	0.2
18:0	7.1	4.8	6.6	7.4	6.9	7.5	5.3	5.1	6.8	6.7	6.7	6.5
18:1 *c*	35.2	30.4	29.3	26.2	27.0	35.5	28.5	26.6	27.8	32.0	25.2	24.8
18:1 t	31.4	6.9	14.3	21.7	13.2	30.8	15.3	13.7	20.1	15.6	22.0	11.4
18:2 *c*	9.0	39.7	33.0	28.8	34.7	9.4	23.3	24.8	30.3	28.8	32.1	44.0
18:2 *tt,ct*	4.1	2.5	2.5	1.1	1.8	3.7	7.0	8.6	1.7	3.4	1.2	0.7
18:3	0.5	2.9	2.8	3.8	4.1	0.7	2.0	2.2	0.5	1.9	0.6	0.8
Others	0.3	1.0	-	0.3	0.7	0.6	1.2	1.8	0.4	0.5	0.3	0.3
TOTAL *trans*/GLC[a]	35.5	9.9	16.8	22.7	15.0	34.5	22.2	22.3	21.8	19.0	23.6	12.4
TOTAL *trans*/IR[b]	35.5	14.3	20.8	25.0	16.5	33.0	22.5	24.3	21.5	23.5	30.0	16.0

[a]Gas chromatography was accomplished with a 20 ft x 1/8 in. SS column packed with 15% OV-275 coated on 100-120 mesh Chromosorb P (AW-DMCS) at 220 C. Injector, 240 C, detector, 300 C; instrument, HP Model 5830; carrier gas flow, 10 ml N_2/min. (0.3 cm/min chart speed).

[b]IR analysis carried out a Beckman IR-7 instrument.

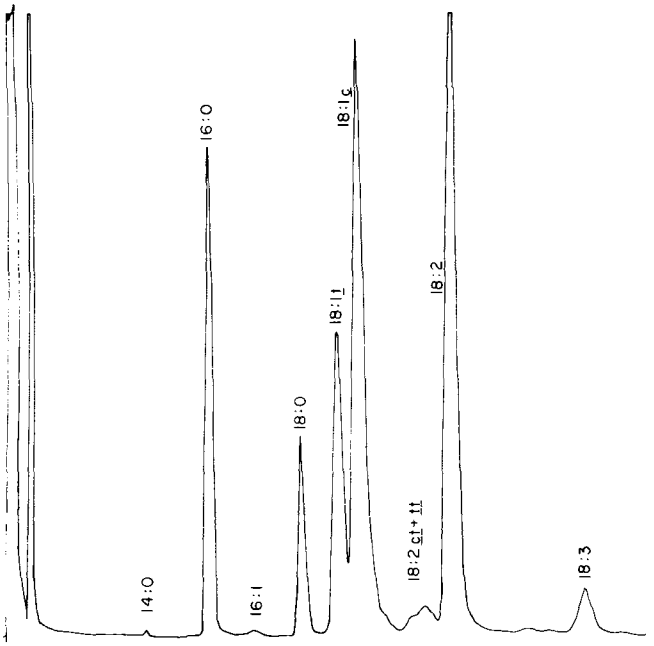

FIG. 2. Separation of margarine methyl ester; (same conditions as Fig. 1).

method. Several standard mixtures of methyl elaidate in methyl oleate varying in *trans* content from 1.0% to 54.3% by wt were prepared. A total of 15 samples were determined over a 2 wk period. The results obtained are tabulated in Table I. Close agreement between the actual weight percent *trans* and that calculated from peak areas was found in all cases regardless of concentration. The absolute error found was about 0.8% when all data were averaged. The relative percent deviation among all values varied from 1% to 10%, with the error increasing as the percent *trans* approached low values. The average percent deviation from the actual value among all concentrations was 4.6%. More accurate results may have been obtained if the instrument operating parameters had been adjusted for the purpose of obtaining optimum response factors for each fatty acid.

The results of analyses carried out on 12 locally purchased, different brands of margarine are presented in Table II. A reproduction of a typical chromatogram of margarine methyl esters is shown in Figure 2. This represents a rather large sample; it illustrates the typical separation of elaidate and oleate obtained. With careful selection of sample size (less than 10 μg) near baseline separation was obtained. The separation of actual margarine esters was always less efficient for elaidate/oleate than was obtained with mixtures of the pure compounds. This is probably due to the mixture of positional/geometrical isomers present in most margarines formed during hydrogenation. It appears that the unresolved doublet always found emerging in front of the linoleate peak is composed of a mixture of *trans-trans* and *trans-cis*-diene, although authentic standards were not available to confirm this. No isomeric peaks corresponding to linolenate isomers were found, although they were probably present in low concentrations. Compared with the results from infrared analysis, those obtained via gas chromatography were (with the exception of one value) within a range of 0.02% to 4.52% of the results obtained with the spectroscopic method. However, the average of all results obtained indicated that the mean deviation between the values obtained with both methods was 2.29 ± 1.7. The overall lower values obtained via the gas chromatographic analysis relative to the infrared values may be caused by the presence of methylene *trans-trans* isomers. The infrared method was designed to measure monoenoic *trans* acids.

TABLE III

Fatty Acid Composition of Body Fluid Lipids (wt %)

	Human milk		Human blood	
Fatty acid	A	B	Serum	Red blood cells
8:0	0.5	0.7	-	-
10:0	2.0	0.7	-	-
12:0	5.4	4.7	-	-
14:0	5.9	6.8	-	-
16:0	19.3	21.2	21.1	22.2
16:1	5.4	3.2	2.0	2.0
18:0	6.4	7.9	7.2	11.9
18:1 *trans*	2.1	4.0	1.9	2.4
18:1 *cis*	34.1	30.1	19.6	19.3
18:2 *trans*	-	-	0.8	0.7
18:2 *cis*	13.5	13.9	36.5	22.0
18:3	1.2	1.0	1.2	0.5
20:4	0.3	0.2	7.9	16.8
Others[a]	3.9	6.4	1.8	2.2

[a]Miscellaneous odd chain and minor components.

A facile analysis of lipids from body fluids for *trans* content would be useful for routine analysis. Accordingly, lipids from human milk, blood serum, and erythrocytes were analyzed. The results from four such analyses are shown in Table III. As expected, small amounts of *trans* unsaturation were present in both milk and serum lipids, as well as in erythrocyte lipids.

The results obtained in the present study indicate that gas chromatographic determination of *trans* unsaturation on columns packed with highly polar stationary phases yields results comparable to those obtained with the spectroscopic method. Results can also be obtained upon very limited quantities of sample, such as those originating from biological sources.

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