Simplified Method for the Determination of *trans* Monoenes in Edible Fats by TLC-GLC

Franz Ulberth* and **Monika Henninger**
Department of Dairy Research and Bacteriology, Agricultural University, A-1180 Vienna, Austria

Several modifications of an established thin-layer chromatography-gas-liquid chromatography (TLC-GLC) procedure for quantitating *trans* unsaturated fatty acids in edible fats are presented. These refinements considerably simplify the procedure without affecting accuracy. The modifications include: i) the use of pre-coated silica sheets, dynamically impregnated with Ag^+, which allow separated bands to be cut off with a pair of scissors; and ii) the use of stearic acid in the deliberately combined saturated and *trans* monounsaturated fatty acid methyl ester bands as an (endogenous) internal standard. *Trans* values thereby obtained agree favorably with the results from the conventional technique.

KEY WORDS: Edible oils, GLC, TLC-argentation chromatography, *trans* fatty acids.

Knowledge of the *trans* fatty acid (FA) content of dietary fats is not only important for the manufacturer as an aid in process optimization (1) but also for health authorities, because unbalanced ingestion of *trans* FA-containing fats may have adverse effects on health (2). Argentation chromatography, either on thin-layer chromatography (TLC) plates or with high-performance liquid chromatography (HPLC) columns, is often recommended as a pre-separation technique for olefinic compounds, and in combination with gas-liquid chromatography (GLC) it enables *trans* FA determinations with high reliability (3,4). Such a TLC-GLC procedure was standardized by IUPAC (5). Unfortunately, this method is laborious and, therefore, not applicable on a routine basis. In this paper we describe several refinements of the basic idea, which lead to a simplified method for an accurate determination of *trans* monounsaturated FA in edible fats.

MATERIALS AND METHODS

Materials. Solvents and chemicals used were of AR-grade quality and purchased from E. Merck (Darmstadt, Germany). Margarines and shortenings were commercial brands and bought in local supermarkets. Standards with an accurately known content of *trans* FA were prepared gravimetrically from soy oil methyl esters and elaidic acid methyl ester (9*t*-18:1) and dissolved in *n*-hexane at a concentration of 20 mg/mL. Heptadecanoic acid methyl ester (17:0) dissolved in *n*-hexane served as an internal standard (IS) in some experiments.

Derivatization. FA methyl esters (FAME) were prepared according to Christopherson and Glass (6).

Argentation-TLC. Argentation-TLC was performed either on self-prepared plates according to Christie (4) or on pre-coated plastic-backed sheets with a 0.2-mm layer of silica gel (Merck Cat. No. 5748) impregnated dynamically with 10% (w/v) $AgNO_3$ in acetonitrile (7). Samples were applied manually with a microliter syringe. Both plates and sheets were developed with *n*-hexane:diethyl ether (9:1).

On the self-prepared plates, spots were visualized with 2′,7′-dichlorofluorescein and recovered as described by Christie (4). For spraying the silica sheets, 0.05% (w/v) rhodamin B in ethanol was used. Spots containing the relevant fractions were cut off with a pair of scissors. Cuttings were inserted in weighing bottles (50 mm o.d. × 30 mm H) and covered with 5 mL diethyl ether. The bottles were allowed to stand for 30 min, while agitating frequently. The resulting extracts were transferred to culture tubes, dried in a stream of N_2, and redissolved in 1 mL isooctane.

GLC. A 0.25 mm × 50 m fused silica capillary column coated with CP-Sil 88 (Chrompack, Middelburg, The Netherlands) was used for separation of FAME. One-μL samples were injected cold on-column. The column was maintained at 105°C for 3 min and temperature programmed at 20°C/min to 140°C (held at this for 1 min) and at 4°C/min to 210°C. Hydrogen at 0.8 bar was the carrier gas. Peak areas were processed by using a computing integrator.

Quantitation of trans *FA by Ag^+/TLC-GLC. Method A* [according to IUPAC 2.208 (5)]: Twenty μL of the *trans*-containing standard solutions or the margarine FAME were applied to a TLC plate and processed as described. One mL of IS solution (20 μg 17:0/mL) was added after scraping off the silica gel. Percentage *trans*-FAME was calculated by (mass of the IS [m_{IS}]/sample mass [m]) × (peak area of *trans* octadecenoates/peak area of the IS).

Method B: Same as method A, but the IS was added before sample application onto the TLC plate.

Method C: Same as method A, but instead of adding an IS the *trans* FAME were scrapped off along with the saturated FAME and the percentage of stearic acid methyl ester (18:0) in the original sample replaces the m_{IS}/m ratio in the original equation.

RESULTS AND DISCUSSION

In IUPAC standard 2.208 (5) a combined GLC-TLC method is described that should be useful for assessing low levels of *trans* unsaturation. Regrettably, this protocol is cumbersome and messy to follow.

In a first attempt to simplify the method, we tried to scale down the sample amount applied to the TLC plate. We found that, instead of 10 mg in IUPAC 2.208, *ca.* 400 μg FAME applied to the plate is by far enough for an accurate determination by GLC of the recovered *trans* FA fraction using on-column injection. However, recovery of 9*t*-18:1 was only 84–89% for standard samples containing the analyte at concentrations between 5.27 and 39.57% (Table 1). The deviations could be largely attributed to an incomplete sample transfer to the self-prepared TLC plate. We observed a considerable "creep back" of sample solution along the syringe needle, resulting in a delivery error. The assumption that incomplete sample transfer is a source of the deviating results was

*To whom correspondence should be addressed at Department of Dairy Research and Bacteriology, Agricultural University, Gregor Mendel-Str. 33, A-1180 Vienna, Austria.

TABLE 1

Accuracy of Different Methods for the Determination of *Trans* Fatty Acids[a]

Actual *trans* content (%)	Found *trans* content (%) by:			
			Method C	
	Method A	Method B	Plate	Sheet
5.27	4.44	5.19	5.16	5.27
12.67	10.95	12.36	12.51	12.46
24.59	21.89	23.74	24.50	24.55
39.57	33.77	39.08	39.07	39.40

[a]Methods A and B were performed with self-prepared TLC plates. For method C, self-prepared plates and pre-coated sheets were used. Mean values of duplicate determinations are reported.

TABLE 2

***Trans* Fatty Acid Content of Various Margarine Oils Assessed by GLC and by the Combined TLC-GLC Method[a]**

Margarine brand	*Trans* content (%)	
	GLC	TLC-GLC
A	9.60	10.82
B	12.92	14.89
C	14.32	19.78
D	14.67	16.72
E	16.46	19.08
F	17.96	21.62
G	18.53	19.57
H	20.64	21.54
I	20.75	25.74
J	30.42	38.05
K	42.05	44.42

[a]Method C in combination with pre-coated silica sheets was used for quantitation of *trans* fatty acids by TLC-GLC. Mean values of at least duplicate analyses are reported.

further validated by method B. With this modification, the IS was carried through the complete procedure and, therefore, errors resulting from sample application, scraping losses, incomplete extraction or overlapping bands of *trans* FAME into saturates were cancelled out. The *trans* FA percentages found agreed well with the known values (Table 1). Although this modification improved substantially the accuracy of the modified IUPAC standard method, the time-consuming weighing steps for preparation of the IS and the test solution still remained.

As a further simplification of the procedure, the standardization method proposed by Christie and Moore (8) was applied. Excellent agreement between the observed and the known *trans* values was found when the simplified method C (Table 1) was used. Precision of method C was tested in separate experiments by applying the method to standards containing 5.27 and 24.59% *trans* FA, respectively. The relative standard deviation (RSD) was 1.15 and 1.62% (n = 4).

Considerable savings of time and labor would result if commercially available pre-coated silica sheets were used instead of the self-prepared TLC plates. In this study, pre-coated plastic-backed silica sheets were impregnated with $AgNO_3$ as described by Aitzetmüller and Guaraldo Goncalves (7) and used for *cis/trans* separation. No differences in chromatographic behavior between self-prepared Ag^+/TLC plates and pre-coated Ag^+/TLC sheets were noticed. Moreover, accuracy of the rapid TLC-GLC method was comparable to the conventional procedure (Table 1). Precision, in terms of the RSD, was 2.91, 1.29, 0.96 and 0.80% at analyte levels of 5.27, 12.67, 24.59 and 29.57% 9*t*-18:1 respectively, in the standard mixtures.

Several brands of margarines and shortenings were analyzed for their *trans* FA content by method C on pre-coated silica sheets (Table 2). Without pre-fractionation, a significant proportion of the total *trans* isomers co-eluted with octadecenoates having the double bond in the *cis* configuration (Fig. 1), thus giving erroneous results when utilizing GLC as the sole separation technique (Table 2).

The approach suggested in this paper is not only equivalent in terms of separating power but also in cost effectiveness, because inexpensive and readily available materials are used. Advantages of the proposed technique are: i) ready-made TLC sheets are used; ii) cutting out the relevant fractions with a pair of scissors is easier than scraping off the spots, thereby avoiding possible cross-contaminations; iii) the lengthy elution procedure is substituted by equilibration of the cuttings in diethyl ether; and iv) rhodamine B is insoluble in diethyl ether, thus rendering washing steps unnecessary.

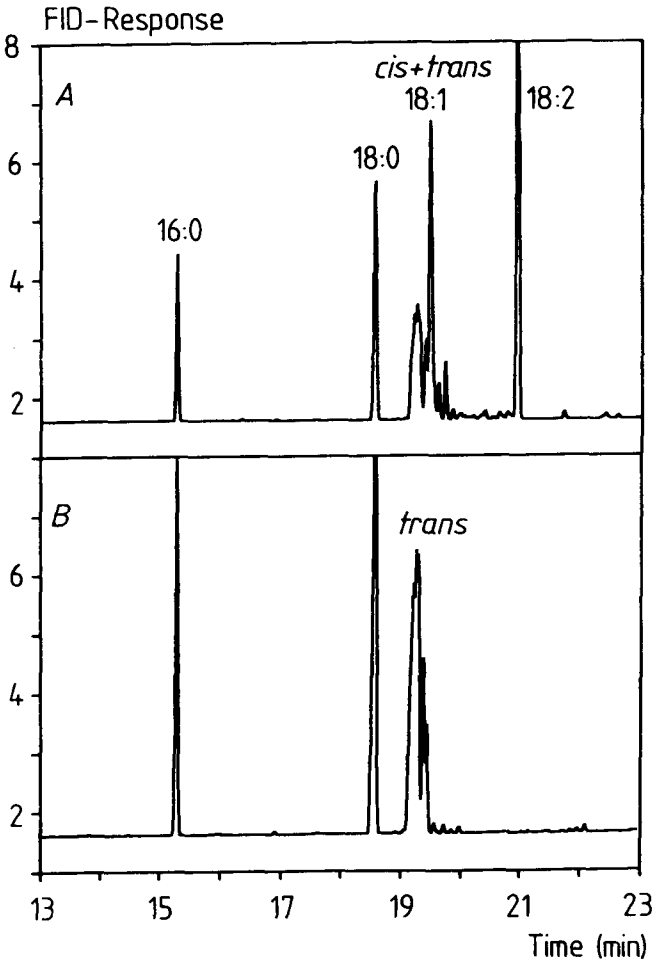

FIG. 1. Separation of *cis/trans* FAME by GLC with a 0.25 mm × 50 m fused silica capillary column coated with CP Sil-88. (*A*) before Ag^+/TLC (original sample); (*B*) after Ag^+/TLC (bands containing saturated and *trans* monoenes).

REFERENCES

1. Patterson, H.B.W., in *Recent Advances in Chemistry and Technology of Fats and Oils*, edited by R.J. Hamilton, and A. Bhati, Elsevier Applied Science, London, United Kingdom, 1987, pp. 41–56.
2. Gurr, M.I., *BNF Nutr. Bull. 11*:105 (1986).
3. Ratnayake W.M.N., and J.L. Beare-Rogers, *J. Chromatogr. Sci. 28*:633 (1990).
4. Christie, W.W., *Lipid Analysis*, Pergamon Press, 2nd edn., Oxford, United Kingdom, 1982.
5. International Union of Pure and Applied Chemistry, *Standard Methods for the Analysis of Oils, Fats and Derivatives*, Blackwell Scientific Publications, Oxford, United Kingdom, 7th edn., 1987.
6. Christopherson, S.W., and R.L. Glass, *J. Dairy Sci. 52*:1289 (1969).
7. Aitzetmüller K., and L.A. Guaraldo Goncalves, *J. Chromatogr. 519*:349 (1990).
8. Christie, W.W., and J.H. Moore, *J. Sci. Fd. Agric. 22*:120 (1971).

[Received October 3, 1991; accepted April 22, 1992]