

Determination of *trans* Fatty Acids in Hydrogenated Vegetable Oils by Attenuated Total Reflection Infrared Spectroscopy: Two Limited Collaborative Studies

M. Adam^a, M. Chew^a, S. Wasserman^a, A. McCollum^a,
R.E. McDonald^b, and M.M. Mossoba^{c,*}

^aLipton, Baltimore, Maryland 21229, ^bFood and Drug Administration, National Center for Food Safety and Technology, Summit-Argo, Illinois 60501, and ^cFood and Drug Administration, Center for Food Safety and Applied Nutrition, Washington, D.C. 20204

ABSTRACT: An attenuated total reflection infrared spectroscopy procedure was collaboratively studied among two sets of five laboratories for quantitating the total *trans* fatty acid levels in neat (without solvent) hydrogenated vegetable oils, measured as triacylglycerols in one study, and as fatty acid methyl ester derivatives in the other. Unlike the fatty acid methyl esters, the triacylglycerols required no derivatization but had to be melted prior to measurement. To obtain a symmetric absorption band at 966 cm⁻¹ on a horizontal background, the single-beam spectrum of the *trans*-containing fat was "ratioed" against that of a refined oil or a reference material that contained only *cis* double bonds. A single-bounce horizontal attenuated total reflection cell that requires 50 µL of undiluted test samples was used for oils, melted fats, or their methyl esters. For fatty acid methyl esters, the reproducibility relative standard deviations were in the range of 0.9 to 18.46% for 39.08 to 3.41% *trans*, determined as methyl elaidate per total fatty acid methyl esters. For five pairs of triacylglycerol blind duplicates, the reproducibility and repeatability relative standard deviations were in the ranges of 1.62 to 18.97%, and 1.52 to 13.26%, respectively, for 39.12 to 1.95% *trans*, determined as trielaidin per total triacylglycerols. Six pairs of spiked triacylglycerol blind duplicates (quality assurance standards) exhibited high accuracy in the range of 0.53 to 40.69% *trans* and averaged a low bias of 1.3%. These statistical analysis results were compared to those collaboratively obtained by the recently adopted AOCS Cd14-95 and AOAC 994.34 Infrared Official Methods. *JAOCS* 75, 353–358 (1998).

KEY WORDS: Attenuated total reflection, collaborative study, infrared, *trans* fatty acids.

The determination of total *trans* fatty acids by infrared spectroscopy (IR) has been widely used for many decades (1). To improve the accuracy of the IR methods (2,3), many modifications have been proposed since the 1960s (4–15), including refinements recently introduced by two new IR Official Meth-

ods that were adopted by the Association of Official Analytical Chemists (AOAC) in 1994 (16) and the American Oil Chemists' Society (AOCS) in 1995 (17). The basis of the IR determination stems from the fact that the C–H out-of-plane deformation band, observed at 966 cm⁻¹, is uniquely characteristic of isolated double bonds with *trans* configuration. These double bonds are found mostly in *trans*-monoenes, and at much lower levels in minor hydrogenation products, such as methylene-interrupted and nonmethylene-interrupted *trans,trans*-dienes, mono-*trans*-dienes, and other *trans*-polyenes. Like most IR procedures, the 1994 AOAC and 1995 AOCS Official Methods are not fully satisfactory because they assume that the band at 966 cm⁻¹ is isolated, while in fact it overlaps with other features of the observed IR spectrum (18). This overlap produces a strongly sloping background that reduces the accuracy of the IR quantitation, particularly at *trans* levels below 5% (16–18).

In 1996, a procedure was proposed (18) to eliminate the highly sloping background by "ratioing" the single-beam spectrum of the hydrogenated fat against that of a *trans*-free reference background material. Thus, a symmetric IR absorption band at 966 cm⁻¹ on a horizontal background was obtained. This "ratioing" of single-beam spectra is equivalent to the differential spectrophotometric procedure proposed for dual-beam dispersive IR instruments by Huang and Firestone in 1974 (7). A second modification was also proposed by Mossoba *et al.* (18); instead of conventional transmission cells, the use of an attenuated total reflection (ATR) IR cell (9,10,19) was used (18) to speed up the determination. With ATR cells, test samples of neat melted fats or fatty acid methyl esters (FAME) are neither weighed nor quantitatively diluted in any solvent. Because it was demonstrated that the "ratioing" and ATR procedure was an acceptable alternative for the IR determination of the total *trans* fatty acid content (18), it was adopted as Recommended Practice Cd 14d-96 by AOCS in 1996 (20). Moreover, in this preliminary investigation (18) no conversion of triacylglycerols (TAG) to FAME was necessary because there were only small differences between *trans* levels in hydrogenated fats and the correspond-

*To whom correspondence should be addressed at the Food and Drug Administration (HFS-717), Center for Food Safety and Applied Nutrition, Washington, DC 20204. E-mail: mmm@vm.cfsan.fda.gov.

ing methyl ester derivatives. To apply this procedure efficiently to the determination of *trans* fat in commercial food products (21), a 50- μ L single-bounce horizontal ATR cell was used instead of a liquid ATR cell (18), which required a much larger quantity of test material (about 1.5–2 mL).

In the present study, the performance of 50- μ L ATR cells was further evaluated in two limited collaborative studies among two sets of five laboratories. The quantitative data were statistically analyzed to determine reproducibility, repeatability, and accuracy of the proposed ATR procedure for the quantitation of *trans* levels in TAG and FAME.

MATERIALS AND METHODS

Lipid standards and reagents were supplied by Nu-Chek-Prep, Inc. (Elysian, MN), Sigma Chemical Co. (St. Louis, MO), and Alltech Associates (Deerfield, IL). All solvents were reagent-grade and were supplied by Aldrich Chemical Co. (Milwaukee, WI). Methyl esters were prepared according to AOAC Official Method 969.33 (22). For the FAME collaborative study, test samples were donated by Dr. W.E. Emmons of SGS Control Services, Inc. (Deer Park, TX); they consisted of five partially hydrogenated soybean oils, a refined bleached deodorized (RBD) soybean oil, and an RBD soybean oil blended with a hydrogenated corn oil. The reference background material was the Sigma reference mixture, AOCS No. 1, which consisted of the methyl esters of palmitic (6.0%), stearic (3.0%), oleic (35.0%), linoleic (50.0%), linolenic (3.0%), and arachidic (3.0%) fatty acids. For the TAG collaborative study, test samples of RBD soybean oil mixed with an industrial blend, and RBD soybean oil spiked with trielaidin (TE), were provided by Lipton (Baltimore, MD). The reference background materials were ultra-degummed bleached expeller soybean oil (Owensboro Grain Co., Owensboro, KY), and RBD soybean oil (Lipton, Baltimore, MD) for the TAG and TE-spiked TAG test samples, respectively.

The affiliations of the five FAME study collaborators were Lipton [Baltimore, two separate quality assurance (QA) laboratories], Food and Drug Administration (FDA) (Washington, DC, and Summit-Argo, IL), and Spectra-Tech (Stamford, CT). The same laboratories participated in the TAG study, except that a collaborator from Mattson Instruments (Madison, WI) replaced the one from Spectra-Tech. The Fourier transform infrared (FTIR) spectrometers Bio-Rad (Cambridge, MA) FTS-60A, Nicolet (Madison, WI) Magna 500, and Perkin-Elmer 2000 (Norwalk, CT) were used by the collaborators at FDA in Washington, DC, Spectra-Tech and Lipton, respectively. Mattson Galaxy 5000 FTIR instruments were used at Mattson Instruments, Lipton, and FDA, IL. Spectra-Tech ZnSe horizontal ATR cells were used by collaborators at FDA (Washington, DC, and Summit-Argo, IL), Spectra-Tech, and Mattson Instruments. A Graseby Specac (Fairfield, CT) ZnSe horizontal ATR cell was used at the two Lipton QA laboratories by different analysts.

The ATR cell required only about 50 μ L of neat (undiluted) test sample without weighing. For the TAG collabora-

tive study, the ATR cell was first warmed to about 65°C by attaching flexible heaters to the top metallic horizontal surface of the ATR cell, whenever melted fats were analyzed. Flexible heaters of model KHLV-0502/10 were acquired from Omega (Stamford, CT) and connected in series to a power transformer (25.2 volt AC, 2-amp), which was purchased from a local outlet. At 4 cm^{-1} resolution, 64 scans were collected. For each observed spectrum, a baseline was drawn between two points, nominally 990 and 945 cm^{-1} , and the area of the 966 cm^{-1} -band (integrated between these same limits) was calculated electronically. At 4 cm^{-1} resolution, the observed minimum points of the *trans* band at 966 cm^{-1} did not always coincide with these integration limits. Differences were observed, particularly at *trans* levels below 5%. At these low levels, the minimum points were typically close to 986 and 947 cm^{-1} , whereas at *trans* levels above 5%, they were near 991 and 941 cm^{-1} . The shifts in the positions of minimum points, particularly at low levels, may be partly due to interferences from conjugated dienes, which absorb near 985 and 950 cm^{-1} . When the observed minima points did not coincide with the nominal points (990 and 945 cm^{-1}), these minima points were usually used to draw the baselines; this is because they were about 4 cm^{-1} away from the nominal points and no distinction was made between these sets of points at the 4 cm^{-1} spectral resolution used in the present collaborative studies. Calibration plots (area vs. percentage *trans*) were generated by ATR for 0.5–50% methyl elaidate (ME) in the ME and methyl oleate (MO) neat reference mixtures, and 0.5–50% TE in the TE and triolein (TO) neat reference mixtures. High correlation coefficients (0.999) were always obtained, which indicated that the variables were sufficiently linear for routine analysis over the given range.

The AOAC (Gaithersburg, MD) program *AOACBUBR* was used for statistical analysis. This program was developed by the AOAC Statistics Committee (23).

RESULTS AND DISCUSSION

Initially, six FAME test samples were collaboratively studied among five laboratories by the “ratioing” and ATR procedure (18). These test samples had been independently analyzed in an interlaboratory study, namely, the 1994 AOCS *trans* Series Smalley Check Sample Program (which validates quality assurance laboratories). The *trans* content (Table 1) of each of the six FAME test samples, which were not blind duplicates, was measured twice by ATR in each of the participating laboratories. The ATR reproducibility standard deviation, $s(R)$, and relative standard deviation, $\text{RSD}(R)$, are plotted against the means for FAME test sample in Figure 1A. A comparison is given in Figure 1B for the $\text{RSD}(R)$ values found for these six FAME test samples by ATR in this study, and by transmission IR (3) in the Smalley study among 11 laboratories. In Figure 2, the $\text{RSD}(R)$ values found by ATR in this study are compared to those obtained by the 1994 AOAC (16) and 1995 AOCS (17) transmission IR Official Methods. Lower $\text{RSD}(R)$ values were found by ATR in each compari-

TABLE 1
Percentage *trans*, Determined as Methyl Elaidate per Total Fatty Acid Methyl Esters (FAME),
for Six FAME Test Samples in Five Laboratories^a

	Lab 1	Lab 1	Lab 2	Lab 2	Lab 3	Lab 3	Lab 4	Lab 4	Lab 5	Lab 5
FAME 3	3.15	3.14	4.50	4.49	3.10	3.50	3.14	3.14	2.96	2.93
FAME 4	8.79	8.82	8.48	8.48	8.70	9.10	8.79	8.75	8.54	8.33
FAME 5	12.71	12.70	12.97	12.90	12.70	12.90	12.68	12.70	12.50	12.38
FAME 1	16.63	16.77	16.58	16.47	16.70	16.90	16.51	16.64	16.18	16.47
FAME 6	23.98	23.94	23.32	23.19	23.50	24.10	24.01	23.99	23.86	23.88
FAME 7	39.15	39.16	38.51	38.64	39.00	38.90	39.20	39.14	39.46	39.60

^aTwo measurements (labeled with the same lab number) were carried out for each test sample in each laboratory.

son. Figure 3 indicates that the ATR means fell between those found in the Smalley study (24) by gas chromatography (GC) (25) and by transmission IR (3).

To evaluate the repeatability of the proposed procedure and to demonstrate that conversion of fats to the corresponding FAME is not necessary, 10 unknowns, consisting of five pairs of TAG blind duplicates, were measured by ATR (after melting the test samples at 65°C) among five laboratories (Table 2). TAG reproducibility $s(r)$ and $RSD(r)$, and repeatability $s(r)$ and $RSD(r)$ are plotted against test sample means in Figure 4A and B, respectively. In Figure 5A, the $RSD(R)$ val-

ues, obtained by ATR for TAG test samples in this study, were generally lower than those found for FAME by the 1994 AOAC (16), but close to those found for FAME by the 1995 AOCS (17) transmission IR Official Methods. The $RSD(R)$ values, obtained for TAG test samples, were generally higher than those reported for FAME test samples in the present ATR studies. Figure 5B compares the pure error, which is a measure of precision (26), for each of the five pairs of TAG blind duplicates, analyzed by ATR in this study, to the lone pair of FAME blind duplicates analyzed by each of the 1994 AOAC

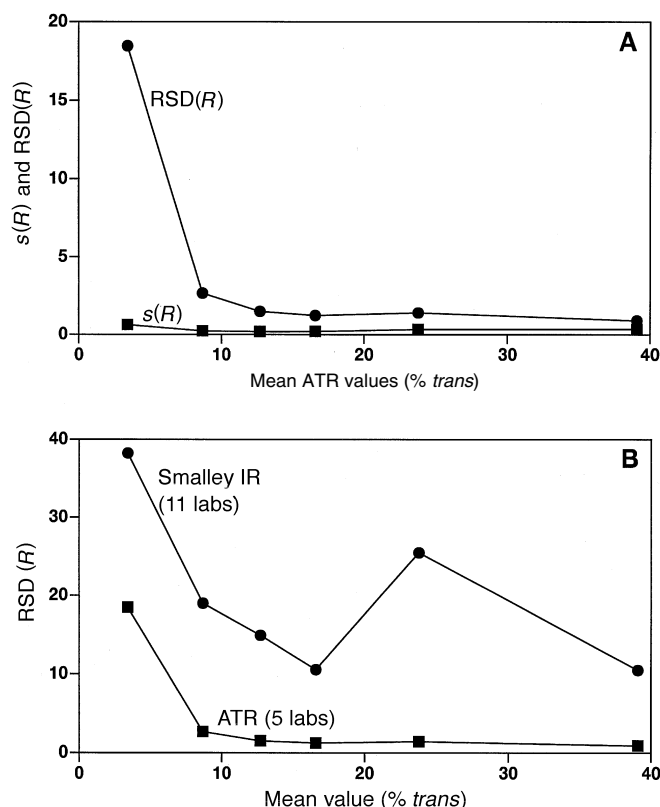


FIG. 1. (A) Plots of reproducibility standard deviation, $s(R)$, and relative standard deviation, $RSD(R)$, against the mean of the *trans* content determined by attenuated total reflection (ATR) as percentage methyl elaidate per total fatty acid methyl esters (FAME); (B) comparison of plots of $RSD(R)$ against the *trans* content mean determined by ATR among five laboratories (■), and by transmission infrared (IR) in the Smalley study among 11 laboratories (23) (●) for the same FAME test samples.

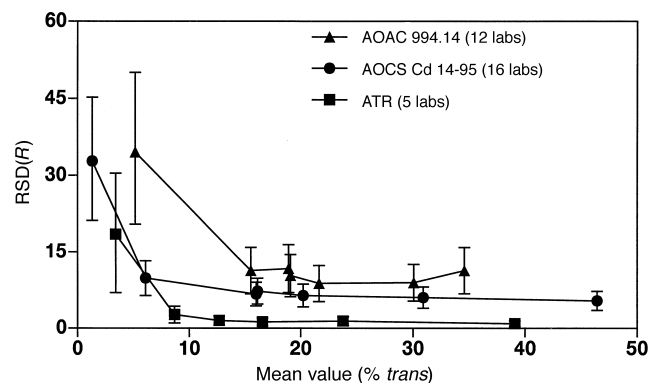


FIG. 2. Comparison of plots of $RSD(R)$ against the mean of the *trans* content determined for FAME by ATR among five laboratories (■), and by the Official AOCS (17) and AOAC (16) transmission IR methods in 16 and 12 laboratories (● and ▲, respectively). For abbreviations see Figure 1. Vertical bars indicate the 95% confidence limits of $RSD(R)$.

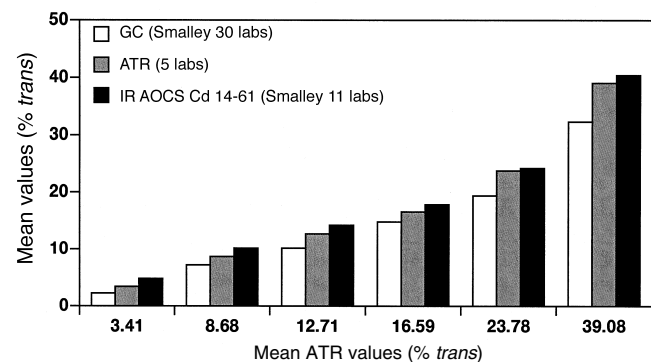


FIG. 3. Comparison of ATR mean (five laboratories) with the Smalley gas chromatographic (GC) and IR means (30 and 11 laboratories, respectively) found for the same FAME test samples. For abbreviations see Figure 1.

TABLE 2
Percentage *trans*, Determined as Triacylglycerols (TAG), for Five Pairs of TAG Blind Duplicate Test Samples in Five Laboratories^a

	Lab 1	Lab 1	Lab 2	Lab 2	Lab 3	Lab 3	Lab 4	Lab 4	Lab 5	Lab 5
TAG 3	1.74	1.73	1.90	2.24	1.96	2.70	2.06	2.11	1.50	1.53
TAG 2	4.03	4.14	3.68	4.15	3.52	3.38	3.70	3.88	2.87	2.99
TAG 5	9.07	9.13	8.66	9.27	7.83	7.22	9.36	9.78	9.02	9.11
TAG 1	24.91	24.75	26.30	26.76	24.32	24.41	26.45	26.6	24.46	24.49
TAG 4	38.12	38.85	39.56	39.60	39.89	38.18	39.60	39.60	39.02	38.78

^aThe data for each pair of TAG blind duplicate test samples are labeled with the corresponding laboratory number.

and 1995 AOCS Official Methods. The highest pure error was found for the 1994 AOAC method. Figure 6A indicates that the TAG ATR means are similar to those obtained for the corresponding FAME by GC (25) in a separate collaborative study among 12 oil producer laboratories (Adam, M., unpublished).

To determine accuracy, 12 QA standards, consisting of six pairs of TAG blind duplicates, were spiked with TE and studied by ATR among five laboratories (Table 3). The spiked TAG reproducibility RSD(*R*) and repeatability RSD(*r*) values were in the range of 3.31 to 17.33 and of 2.99 to 17.24%, respectively, for 40.31 to 0.53% *trans*, determined as TE per total TAG. Figure 6B compares the true values (based on the

amount of TE added gravimetrically) for each of the six pairs of TAG QA standard blind duplicates to those determined by ATR. Accuracy was high and averaged a low bias of 1.3%.

The adverse impact of a highly sloping spectral background on accuracy was successfully eliminated by "ratioing out" the overlapping broad features that caused it. The use of an ATR cell added speed and convenience to the procedure because weighing test portions and quantitatively diluting them in CS₂ were no longer required. The requirement to convert TAG to FAME was also eliminated; however, TAG required melting prior to and during ATR measurement. Satis-

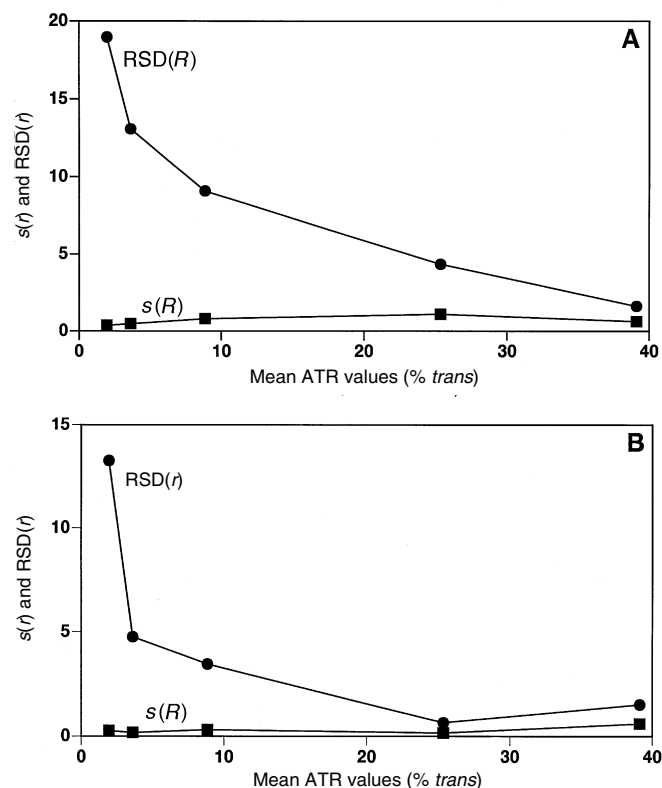


FIG. 4. (A) Plots of reproducibility standard deviation, *s*(*R*), and relative standard deviation, RSD(*R*), against the mean of the *trans* content determined by ATR among five laboratories as percentage trielaidin (%TE) per total triacylglycerols (TAG); (B) plots of repeatability standard deviation, *s*(*r*), and relative standard deviation, RSD(*r*), against the mean of the *trans* content determined by ATR among five laboratories as %TE per total TAG. For abbreviations see Figure 1.

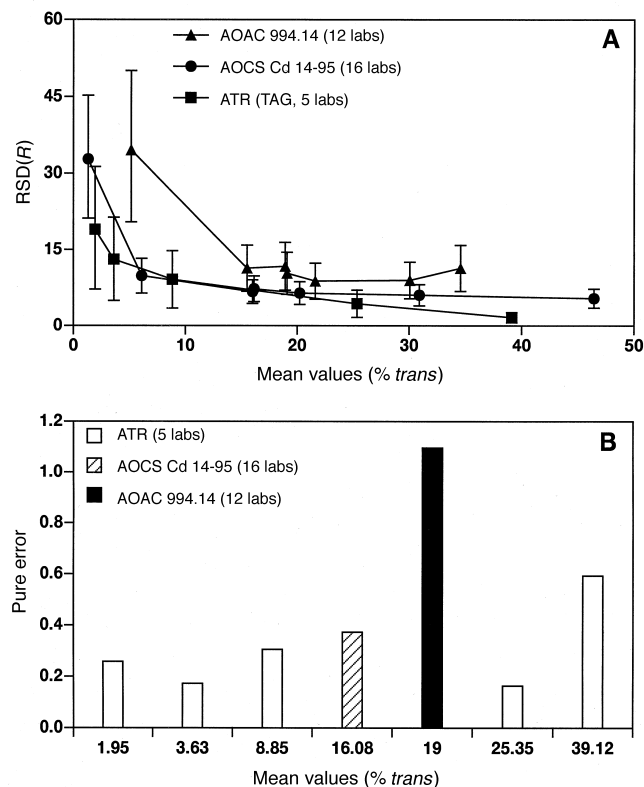


FIG. 5. (A) Comparison of plots of RSD(*R*) against the *trans* content mean determined for TAG by ATR among five laboratories (■), and for FAME by the official AOCS (17) and AOAC (16) transmission IR methods in 16 and 12 laboratories (● and ▲, respectively); (B) comparison of pure error found for the five pairs of TAG blind duplicates determined by ATR among five laboratories, and for a pair of FAME blind duplicates determined by each of the official AOCS and AOAC transmission IR methods in 16 and 12 laboratories, respectively. For abbreviations see Figures 1 and 4.

TABLE 3

Percentage *trans*, Determined as Trielaidin per Total Triacylglycerols (TAG), for Six Pairs of Spiked TAG Blind Duplicate Test Samples in Five Laboratories^a

	Lab 1	Lab 1	Lab 2	Lab 2	Lab 3	Lab 3	Lab 4	Lab 4	Lab 5	Lab 5
SPK 1	0.52	0.44	0.55	0.39	0.62	0.50	0.72	0.53	0.50	0.50
SPK 2	1.08	0.92	1.04	0.88	1.10	1.10	1.20	1.10	0.83	0.73
SPK 3	4.05	4.27	3.98	4.10	4.26	4.32	4.24	4.20	3.73	3.65
SPK 5	10.57	10.78	10.66	10.44	^b	^b	11.16	12.26	11.05	10.98
SPK 6	22.91	23.67	22.89	23.67	27.81	23.17	23.49	23.39	23.04	22.98
SPK 4	40.49	39.95	40.08	40.75	39.77	43.47	40.42	40.63	38.66	38.83

^aThe data for each pair of spiked TAG blind duplicate test samples are labeled with the corresponding laboratory number.

^bNot done.

factory reproducibility, repeatability, and accuracy were demonstrated in the present collaborative studies. No major difference in reproducibility was found between the FAME and the TAG studies. The reproducibility relative standard deviation values, RSD(R), found for FAME in the present ATR studies were lower than those reported for the 1994 AOAC (16) and 1995 AOCS (17) transmission Official Methods.

ACKNOWLEDGMENTS

The authors thank D. Firestone for guidance and discussions during the course of this work. The authors acknowledge R. Simler (Spectra-Tech) and P. Ruer (Mattson Instruments) for participating in the

FAME and TAG collaborative studies, respectively. Graseby Specac and Mattson Instruments generously provided technical assistance to the Lipton QA group (Baltimore, MD). Bio-Rad (Digilab Division) generously loaned the FDA (Summit-Argo, IL) an ATR cell for the duration of the studies. The assistance of R. Sirpak (Graseby Specac); J. Anderson and T. Wright (Mattson Instruments); J. Powell, S. Cantor, and P. Littlefield (Bio-Rad); W. Wihlborg and E. Manke, Jr. (Spectra-Tech); and S. Cianci, M. Meltzer, F. McClure, and R. Newell (FDA) is appreciated.

REFERENCES

1. Firestone, D., and A. Sheppard, Determination of *trans* Fatty Acids, in *Advances in Lipid Methodology-One*, edited by W.W. Christie, The Oily Press, Ayr, 1992, pp. 273–322.
2. AOAC, Association of Official Analytical Chemists, *Official Methods of Analysis*, edited by K. Helrich, Gaithersburg, 1990, Method 965.34.
3. AOCS, American Oil Chemists' Society, *Official Methods and Recommended Practices*, edited by D. Firestone, Champaign, 1993, Official Method Cd 14-61.
4. AOAC, Association of Official Analytical Chemists, *Official Methods of Analysis*, edited by K. Helrich, Gaithersburg, 1994, Method 994.14.
5. IUPAC, International Union of Pure and Applied Chemistry, *Standard Methods for the Analysis of Oils, Fats and Derivatives*, edited by C. Paquot and A. Hautfenne, Blackwell Scientific Publications, Oxford, 1987, Method 2.207.
6. Firestone, D., and P. LaBouliere, Determination of Isolated *Trans* Isomers by Infrared Spectrophotometry, *J. Assoc. Off. Anal. Chem.* 48:437–443 (1965).
7. Huang, A., and D. Firestone, Determination of a Low Level Isolated *Trans* Isomers in Vegetable Oils and Derived Methyl Esters by Differential Infrared Spectrophotometry, *Ibid.* 54:47–51 (1971).
8. Allen, R.R., The Determination of *Trans* Isomers in GLC Fractions of Unsaturated Esters, *Lipids* 4:627–628 (1969).
9. Belton, P.S., R.H. Wilson, H. Sadeghi-Jorabchi, and K.E. Peers, A Rapid Method for the Estimation of Isolated *Trans* Double Bonds in Oils and Fats Using FTIR Combined with ATR, *Lebensm. Wiss. Technol.* 21:153–157 (1988).
10. Dutton, H.J., Analysis and Monitoring of *Trans*-Isomerization by IR ATR Spectrophotometry, *J. Am. Oil Chem. Soc.* 51:406–409 (1974).
11. Sleeter, R.T., and M.G. Matlock, Automated Quantitative Analysis of Isolated (Nonconjugated) *Trans* Isomers Using FTIR Spectroscopy Incorporating Improvements in the Procedure, *Ibid.* 66:121–127 (1989).
12. Ulberth, F., and H.-J. Haider, Determination of Low Level *Trans* Unsaturation in Fats by FTIR Spectroscopy, *J. Food Sci.* 57:1444–1447 (1992).
13. Madison, B.L., R.A. DePalma, and R.P. D'Alonzo, Accurate

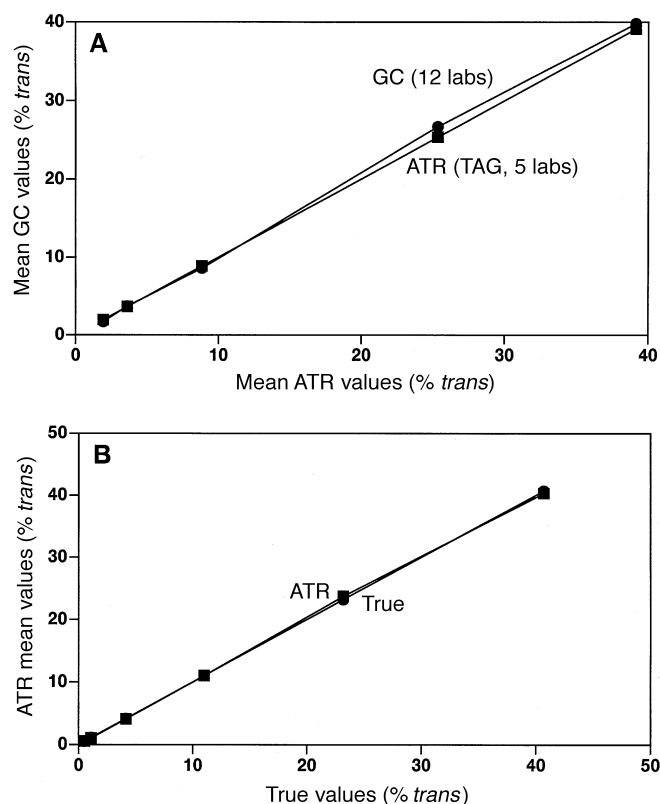


FIG. 6. (A) Comparison of ATR means (five laboratories) for five pairs of TAG blind duplicates with GC means (12 laboratories) found for the corresponding FAME test samples; (B) comparison of ATR means (five laboratories) for six pairs of spiked TAG quality assurance blind duplicate standards with the True value (determined gravimetrically). For abbreviations see Figures 1, 3, and 4.

- Determination of *Trans* Isomers in Shortenings and Edible Oils by Infrared Spectrophotometry, *J. Am. Oil Chem. Soc.* 59: 178–181 (1982).
14. Lanser, A.C., and E.A. Emken, Comparison of FTIR and Capillary GC Methods for Quantitation of *Trans* Unsaturation in Fatty Acid Methyl Esters, *Ibid.* 65:1483–1487 (1988).
 15. Toschi, T.G., P. Capella, C. Holt, and W.W. Christie, A Comparison of Silver Ion HPLC Plus GC with FTIR Spectroscopy for the Determination of *Trans* Double Bonds in Unsaturated Fatty Acids, *J. Sci. Food Agric.* 61:261–266 (1993).
 16. AOAC, Association of Official Analytical Chemists, *Official Method of Analysis*, edited by K. Helrich, Gaithersburg, 1994, Method 994.14.
 17. AOCS, American Oil Chemists' Society, *Official Methods and Recommended Practices*, edited by D. Firestone, Champaign, 1995, Official Method Cd 14-95.
 18. Mossoba, M.M., M.P. Yurawecz, and R.E. McDonald, Rapid Determination of the Total *Trans* Content of Neat Hydrogenated Oils by Attenuated Total Reflection Spectroscopy, *J. Am. Oil Chem. Soc.* 73:1003–1009 (1996).
 19. Mirabella, F.M., Jr. (ed.), *Internal Reflection Spectroscopy, Practical Spectroscopy Series*, Marcel Dekker, New York, 1992, Vol. 15.
 20. AOCS, American Oil Chemists' Society, *Official Methods and Recommended Practices*, edited by D. Firestone, Champaign, 1997, Recommended Practice Cd 14d-96.
 21. Ali, L.H., G. Angyal, C.M. Weaver, J.I. Rader, and M.M. Mossoba, Determination of Total *Trans* Fatty Acids in Foods: Comparison of Capillary-Column Gas Chromatography and Single-Bounce Horizontal Attenuated Total Reflection Infrared Spectroscopy, *J. Am. Oil Chem.* 73:1699–1705 (1996).
 22. AOAC, Association of Official Analytical Chemists, *Official Methods of Analysis*, edited by K. Helrich, Gaithersburg, 1994, Method 969.33.
 23. Guidelines for Collaborative Study Procedure to Validate Characteristics of a Method of Analysis, *J. Assoc. Off. Anal. Chem.* 72:694–704 (1989).
 24. AOCS, *Smalley Series Report*, American Oil Chemists' Society, Champaign, 1995.
 25. AOCS, *Official Methods and Recommended Practices*, edited by D. Firestone, American Oil Chemists' Society, Champaign, 1993, Official Method Ce 1c-89.
 26. Draper, N.R., and H. Smith, *Applied Regression Analysis*, John Wiley & Sons, New York, 1981, pp. 33–42.

[Received July 23, 1997; accepted December 17, 1997]