Comparison of Attenuated Total Reflection Infrared Spectroscopy to Capillary Gas Chromatography for *trans* Fatty Acid Determination

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ABSTRACT: Gas chromatography (GC) has been a standard analytical tool in lipid chemistry. The rapid attenuated total reflection (ATR) infrared (IR) American Oil Chemists' Society (AOCS) *Recommended Practice* (Cd 14d-97) was compared to the capillary GC AOCS *Recommended Practice* (Ce 1f-97) that was optimized to accurately determine total *trans* fatty acids on highly polar stationary phases. This comparative evaluation was validated in an independent laboratory. These procedures were used to quantitate the total *trans* fatty acid levels in partially hydrogenated vegetable oils, measured as neat (without solvent) triacylglycerols (TAG) by ATR and as fatty acid methyl ester (FAME) derivatives by capillary GC. Unlike FAME, TAG determination by ATR required no derivatization, but samples had to be melted prior to measurement. Five blind replicates for each of three accuracy standards and three test samples were analyzed by each technique. The GC and ATR determinations were in good agreement. Accuracy was generally high. The ratios of ATR mean *trans* values (reported as percentage of total TAG) to the true values (based on the amount of trielaidin added gravimetrically) were 0.89, 0.98, and 1.02 for accuracy standards having about 1, 10, and 40% *trans* levels. The corresponding GC values, determined as percentage of total FAME, were 0.98, 0.99 and 1.04. The ratios of mean *trans* values determined by these techniques were ATR/GC 0.85, 1.04, and 1.01 for test samples having *trans* levels of about 0.7, 8, and 38%, respectively. The optimized GC procedure also minimized the expected low bias in *trans* values due to GC peak overlap found with the GC *Official Method* Ce 1c-89. Satisfactory repeatability and reproducibility were obtained by both ATR and GC.

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For several decades analytical methods, such as gas chromatography (GC) (1) and infrared (IR) spectroscopy (2,3), for the determination of total *trans* fatty acids in partially hydrogenated vegetable oils have each been widely used (4) and repeatedly modified in order to improve accuracy (5,6). A recent publication by Duchateau *et al.* (5) detailed optimized capillary GC separations on highly polar stationary phases for the accurate determination of *trans* fatty acids in partially hydrogenated vegetable oils and in refined (deodorized or stripped) oils. This procedure was adopted by the American Oil Chemists' Society (AOCS) as *Official Method* AOCS Ce 1f-96 (7). The GC *Official Method* AOCS Ce 1c-89 (1) for partially hydrogenated vegetable oils underestimates some *trans* C_{18} monoene (18:1) positional isomers in favor of *cis* 18:1 isomers, with which they overlap. Two relatively minor peaks attributed to the *trans*-12 *and trans*-13 plus *trans*-14 18:1 isomers were resolved by the optimized GC procedure (5), and this reportedly improved the accuracy of the GC determination. These two minor peaks (labeled "valley peaks") emerged between those of the major *trans* 18:1 isomers and the intense *cis*-9 18:1 peak. In refined vegetable oils mono-*trans* 18:2 and 18:3 isomers occur at levels that can reach 1–3% of total fat. The optimized GC procedure also eliminated the overlap between the *trans*-9,*cis*-12,*cis*-15-18:3 and the *cis*-11 20:1 peaks (5).

In publications on the determination of total *trans* fatty acids, GC and IR data were often compared (4), and *trans* levels determined by GC were usually found to be lower than those obtained by IR spectroscopy (8). On the other hand, IR *Official Methods,* such as AOAC 994.14 (2) and AOCS Cd 14-95 (3), are not fully satisfactory because they lead to relatively higher *trans* levels, particularly below 5%. They are based on measuring the C-H out-of-plane deformation band observed at 966 cm^{-1}, which is uniquely characteristic of isolated (nonconjugated) double bonds with *trans* configuration. Unfortunately, this absorption overlaps with other bands in the IR spectra of FAME or TAG, resulting in a strongly sloping background (2,3,6) that reduces the accuracy of the IR quantitation. Of the many refinements proposed to date (4,6,8–10), the one by Mossoba *et al.* (6) described a modified IR procedure that could overcome this problem. With this procedure the highly sloping background was eliminated by "ratioing" the single-beam spectrum of the partially hydrogenated fat against that of a *trans*-free reference background material. Thus, a symmetrical IR absorption band at 966 cm^{-1} on a horizontal background was obtained, which gave rise to improved accuracy. This modified IR procedure (6) was also rapid (5

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min) because an attenuated total reflection (ATR) liquid cell (capacity 1.5–2 mL) was used that required neither weighing nor quantitative dilution of neat melted fat test samples in any solvent. By using a single-bounce horizontal ATR cell with 50 µL capacity, this ATR IR procedure was optimized and applied to the determination of total *trans* fatty acids in commercial food products (11), validated in two limited collaborative studies (12), and adopted as *Recommended Practice* AOCS Cd 14d-97 (13). Attempts were abandoned to use a single-µL ATR cell for the quantitation of *trans* fatty acids in biological matrices (14,15) due to spectral interferences from conjugated fatty acids. Recently, Sedman *et al.* (8) used the ATR IR *Recommended Practice* AOCS Cd 14d-97 (13) to validate a transmission IR procedure they developed that was designed to simultaneously determine *trans* content, *cis* content, iodine value, and saponification number for neat fats and oils. Using their proposed procedure, they analyzed more than 100 partially hydrogenated rapeseed and soybean oils and reported excellent concurrence of *trans* levels with those obtained by the ATR IR *Recommended Practice* AOCS Cd 14d-97 (13); the calculated mean difference was 0.6% *trans*. They also determined the *trans* content by using the GC *Official Method* AOCS Ce 1c-89 (1) for partially hydrogenated vegetable oils. Due to the overlap of some GC peaks for *trans* and *cis* 18:1 isomers, the reported GC values were predictably lower than those found by the two IR procedures (8).

In the present study, the performance of the *Recommended Practice* AOCS Cd 14d-97 (13) for the determination of total *trans* fatty acids by the rapid ATR IR spectroscopic procedure was compared to that of the optimized capillary GC procedure AOCS Ce 1f-97 (7). This comparison was peer-verified in an independent laboratory. The quantitative data were statistically analyzed in order to determine and compare the reproducibility, repeatability, and, in particular, accuracy of these procedures for the quantitation of total *trans* fatty acids as TAG by ATR IR spectroscopy and as FAME by GC.

TABLE 1
Total *trans* FAME (as percentage of total FAME) by GC, and Total *trans* TAG (as percentage of total TAG) by ATR for Accuracy Standards (ACC1, ACC2, and ACC3)[a]

	Accuracy standards	
	GC	ATR
ACC1		
LAB 1[b]	1.10, 1.15, 1.10, 1.08, 1.14	1.16, 1.05, 1.03, 1.02, 1.03
LAB 2[b]	1.20, 1.19, 1.23, 1.17, 1.38	1.09, 1.09, 1.08, 1.12, 1.04
TRUE[c]	1.20	1.20
XBAR[d]	1.17	1.07
s(*r*)[e]	0.06	0.05
s(*R*)	0.1	0.05
RSD(*r*)[f]	5.39	4.28
RSD(*R*)	8.69	4.28
ACC2	GC	ATR
LAB 1	10.29, 10.23, 10.21, 10.29, 10.24	10.33, 10.44, 10.35, 10.23, 10.23
LAB 2	10.14, 10.14, 10.31, 10.13, 10.11	9.58, 9.65, 9.86, 10.51, 9.75
TRUE	10.31	10.31
XBAR	10.21	10.09
s(*r*)	0.06	0.27
s(*R*)	0.08	0.4
RSD(*r*)	0.62	2.69
RSD(*R*)	0.81	3.94
ACC3	GC	ATR
LAB 1	40.51, 40.54, 40.51, 40.50, 40.50	40.94, 40.93, 40.18, 40.84, 40.87
LAB 2	42.15, 41.96, 41.56, 41.36, 41.09	39.57, 39.00, 39.61, 41.00, 39.05
TRUE	39.56	39.56
XBAR	41.07	40.2
s(*r*)	0.31	0.62
s(*R*)	0.83	0.96
RSD(*r*)	0.75	1.53
RSD(*R*)	2.03	2.39

[a]FAME, fatty acid methyl esters; GC, gas chromatography; TAG, triacylglycerols; ATR, attenuated total reflection.
[b]LAB1, Lipton laboratory; LAB2, Perdue Farms laboratory.
[c]TRUE, determined gravimetrically.
[d]XBAR, the overall mean.
[e]*s*(*r*) and *s*(*R*), repeatability and reproducibility standard deviations, respectively.
[f]RSD(*r*) and RSD(R), repeatability and reproducibility relative standard deviations, respectively.

TABLE 2
Total *trans* FAME (as percentage of total FAME) by GC, and Total *trans* TAG (as percentage of total TAG) by ATR for Test Samples (UNK1, UNK2, and UNK3)[a]

	Test samples	
	GC	ATR
UNK1		
LAB 1	0.79, 0.67, 0.72, 0.78, 0.69	0.79, 0.62, 0.62, 0.67, 0.63
LAB 2	0.64, 0.81, 0.69, 0.96	0.64, 0.64, 0.61, 0.60, 0.60
XBAR	0.75	0.64
s(*r*)	0.1	0.05
s(*R*)	0.1	0.06
RSD(*r*)	13.51	8.28
RSD(*R*)	13.51	9.1
UNK2	GC	ATR
LAB 1	7.78, 7.37, 8.00, 7.89, 7.93	8.82, 9.01, 8.89, 8.70, 8.72
LAB 2	8.93, 8.24, 8.88, 9.10, 8.37	8.34, 8.34, 8.30, 8.49, 8.19
XBAR	8.25	8.58
s(*r*)	0.32	0.12
s(*R*)	0.7	0.34
RSD(*r*)	3.87	1.38
RSD(*R*)	8.53	4.27
UNK3	GC	ATR
LAB 1	37.71, 37.69, 37.92, 38.15, 37.95	38.89, 39.38, 39.45, 38.85, 38.86
XBAR	37.73	38.19
s(*r*)	0.17	0.21
s(*R*)	0.26	1.29
RSD(*r*)	0.46	0.56
RSD(*R*)	0.7	3.37

[a]See Table 1 for abbreviations.

MATERIALS AND METHODS

Lipid standards and reagents were obtained from Nu-Chek-Prep, Inc. (Elysian, MN), Sigma Chemical Co. (St. Louis, MO), and Alltech Associates (Deerfield, IL). All solvents were reagent grade and were purchased from Aldrich Chemical Co. (Milwaukee, WI). Methyl esters were prepared according to AOAC Official Method 969.33 (16). TAG test samples consisted of refined bleached deodorized soybean oil mixed with industrial blends containing various levels of *trans* fat. The reference background material used was the ultra degummed bleached expeller soybean oil (Owensboro Grain Co., Owensboro, KY). Accuracy standards were prepared by spiking this oil with trielaidin (TE).

Hewlett-Packard (Avondale, PA) gas chromatographs model 5890 Series II and Mattson (Madison, WI) Galaxy 5000 Fourier transform infrared (FTIR) spectrometers equipped with Graseby Specac (Fairfield, CT) ZnSe horizontal ATR cells were used by the submitting (Lipton, Baltimore, MD) and peer (Perdue, Salisbury, MD) laboratories.

The *Recommended Practices* AOCS Cd 14d-97 (13), for the rapid determination of total *trans* TAG by ATR IR spectroscopy, and AOCS Ce 1f-97 (7), for the determination of total *trans* FAME by GC on 50 m × 22 mm (i.d.) CP Sil 88 (Chrompack, Bridgewater, NJ) capillary columns with a 0.19 μm stationary phase, were followed. As recently detailed by Adam *et al.* (12), the single-bounce horizontal ATR cell required only about 50 μL of neat (undiluted) test sample without weighing. For TAG test samples, the ATR cell was first warmed to about 68–70°C by attaching flexible heaters to the top metallic horizontal surface of the ATR cell whenever melted fats were analyzed. Flexible heaters model KHLV-0502/10 were acquired from Omega (Stamford, CT) and connected in series to a power transformer (25.2 V AC, 2-A) 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 (12). Calibration plots (area vs. percent *trans*) were generated by ATR for 0.5–50% TE in the reference mixtures. High correlation coefficients (0.999) were always obtained. The AOAC (Gaithersburg, MD) program AOACBUBR was used for statistical analysis. This program was developed by the AOAC Statistics Committee (17).

RESULTS AND DISCUSSION

In order to compare the accuracy, repeatability (*r*), and reproducibility (*R*) of the rapid ATR *Recommended Practice* AOCS Cd 14d-97 to those of the optimized GC *Recommended Practice* AOCS Ce 1f-97 (7), three accuracy standards each consisting of five blind replicates were measured as TAG by ATR and as FAME by GC in the submitting and peer laboratories. Data on accuracy, reproducibility *s*(*R*) and

RSD(R), and repeatability $s(r)$ and RSD(r) (where s is standard deviation and RSD is relative standard deviation) are presented in Table 1. A comparison of the true (based on the amount of TE added gravimetrically) *trans* values at the three levels investigated, 1.20, 10.31, and 39.56% (reported as percentage of total fat), and those found by GC indicated that accuracy of the optimized GC procedure was generally high. Specifically, the values for "GC as percent of true" were 98, 99, and 104 for the three levels investigated in increasing order, respectively. The corresponding "ATR as percent of true" values were 89, 98, and 102 and indicated that accuracy was better by GC than by ATR at 1.20 and 10.31% *trans*, but the opposite was found at 39.56% *trans*. On the other hand, both RSD(r) and RSD(R) values obtained for TAG test samples (ATR) were lower than those reported for FAME test samples (GC) at 1.20% *trans*, while the reverse was found at 10.31 and 39.56% *trans* (Table 1). Similarly, three unknown test samples, each consisting of five blind replicates, were measured as FAME by GC and as TAG by ATR in the submitting and peer laboratories. The mean GC values were 0.75, 8.25, and 37.73% *trans* (Table 2). In general, small differences in the overall mean *trans* values were found between GC and ATR. At the two higher levels (GC means 8.25 and 37.73%, ATR means 8.58 and 38.19%), the "GC as percent of ATR" values were 96 and 99, respectively. However, the corresponding difference was larger, 117, at the lowest *trans* level investigated (GC mean 0.75%, ATR mean 0.65%). One might attribute this larger difference to the fact that the *trans* level in this case was below 0.8%, the lower limit of quantitation by ATR according to *Recommended Practice* AOCS Cd 14d-97 (13). However, at this *trans* level, both RSD(r) and RSD(R) values obtained by ATR for TAG test samples were lower (near 9%) than those found by GC (near 13%) for FAME test samples (Table 2). Only small differences in RSD(r) and RSD(R) were found at the higher two levels.

Satisfactory accuracy, reproducibility, and repeatability were demonstrated in the present peer-verified comparison, and no major differences in these parameters were found between the FAME and the TAG test samples. The ATR IR *Recommended Practice* AOCS Cd 14d-97 (13) was rapid (5 min) and convenient because weighing TAG test portions and quantitatively diluting them in CS_2 were no longer required, while the optimized GC procedure required converting TAG to FAME. However, TAG required melting prior to and during ATR measurement. On the other hand, GC is most useful for the determination of the complete fatty acid composition. The improved GC resolution obtained with *Recommended Practice* AOCS Ce 1f-97 (7) has resulted in high accuracy and eliminated the low bias usually found by GC relative to IR methods.

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