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ORIGINAL PAPER

Regulatory Infrared Spectroscopic Method for the Rapid Determination of Total Isolated *Trans* Fat: A Collaborative Study

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Abstract Using attenuated total reflection-Fourier transform infrared (ATR-FTIR) spectroscopy, collaborating scientists in ten different laboratories measured (in duplicate) the total *trans* fat content of ten fat or oil test samples, two of which were blind duplicates. The procedure used entailed measuring the height of the negative second derivative of the IR absorption band at 966 cm^{-1}. This absorption is attributed to the C–H deformation vibration that is characteristic of isolated (non-conjugated) double bonds with the *trans* configuration. The precision of ATR-FTIR results in this international collaborative study was satisfactory and led to the approval of this validated procedure as official method AOCS Cd 14e-09 in late 2009. This official method is also suitable for analysis of total isolated *trans* fat and oil products containing, or supplemented with, *trans* conjugated linoleic acid (CLA) isomers. Although this method does not require derivatization of the oil or fat test materials, as required for GC, fats and oils in foods must be extracted with organic solvents before analysis. This method is also rapid (5 min) and does not require any weighing or quantitative dilution of unknown neat fat or oil test samples in any solvent. The AOCS Cd 14e-09 method is suitable for determination of test samples with zero *trans* fat, which is defined according to the US labeling regulations as 0.5 g *trans* fat per serving or 1.8% *trans* fat, as a percentage of total fat.

Keywords Fats and oils · Infrared · Spectroscopy · Lipid chemistry · Lipid analysis

Abbreviations

FT	Fourier transform
IR	Infrared
ATR	Attenuated total reflection

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PHCO Partially hydrogenated canola oil
TE Trielaidin
TP Tripalmitin

Introduction

The rapid (5 min) determination of total isolated *trans* fatty acids by infrared (IR) spectroscopy has been a widely used standard procedure [1, 2]. This method is based on measurement of the height or area under the 966 cm^{-1} C–H out-of-plane deformation band, which is uniquely characteristic of isolated (non-conjugated) double bonds with the *trans* configuration (Figs. 1, 2).

Determination of total isolated *trans* fats by IR spectroscopy is negatively affected at levels below 5%, as a percentage of total fat. This is because the baseline offset and slope near the *trans* absorption frequency (Figs. 1, 2) results in high variability of measurement precision [1, 2]. For instance, for test samples with reported IR means of 1.0, 1.5, and 1.7% *trans* (as a percentage of total fat), the reproducibility, as relative standard deviation $(RSD)_R$, values were found to be high, namely 29.3, 12.5, and 23.7%, respectively [1, 2]. Therefore, it was important to develop a new IR method that improved the precision in the range of approximately 1–5% *trans* fat, as a percentage of total fat. It is noted that the 1999 and 2000 IR official methods [1, 2] are only adequate for the determination of *trans* fat at levels above 5% (as a percentage of total fat).

To overcome the adverse effect of the sloping baseline and possible additional absorption interferences found in *trans* fat spectra, a newly developed IR procedure [3–6] was validated in this international collaborative study. It entails measurement of the height of the negative second derivative of the total isolated *trans* absorption band at 966 cm^{-1} (Figs. 2, 3). A second derivative has traditionally been used to enhance spectral features [3]. Advantages of measuring the second derivative of the *trans* absorption band include: first, problems associated with the baseline offset and slope no longer exist. Second, the height of the negative second derivative is easy to measure from the horizontal line (Fig. 2), and is directly proportional to the amount of total isolated *trans* fat in a test sample. Third, a second derivative has a narrower bandwidth than an absorption band, which therefore enables the detection of interference bands that are adjacent to the 966 cm^{-1} band of interest. For example, interference bands were observed for coconut oil and cocoa butter when using the ATR-FTIR method [4–6], but no detectable bands were found at 966 cm^{-1} in the second-derivative spectra. The maxima at slightly lower frequencies, near 960 cm^{-1} (Fig. 4), were due to high levels of saturated fat but negligible (0.1%) levels of *trans* fat [7]. Because of the close proximity of these IR bands to 966 cm^{-1} in the ATR-FTIR second-derivative spectra, they could be erroneously attributed to isolated *trans* fatty acids [4]. Fourth, second-derivative spectra are suitable for determination of total isolated *trans* fat in the presence of conjugated *cis*/*trans* and *trans*/*trans* conjugated linoleic acid (CLA) isomers. The narrow bandwidth of the second-derivative band enables the spectral resolution of the 966 cm^{-1} band from those due to *trans* CLA isomers near 990 cm^{-1} (*trans*/*trans*) and the doublet near 985 and 945 cm^{-1} (*cis*/*trans*) [8].

In order to meet *trans* fat labeling requirements, in particular the claim of zero gram *trans* fat per serving [9, 10], it was necessary to develop a method with sensitivity that is capable of measuring 0.5 g *trans* fat per serving. A recently published compositional database on the *trans* fat content of a wide range of foods [11] was used to estimate the *trans* fat content, as a percentage of total fat. For a food product that consists mostly of fat or oil, for example Ranch dressing with 27.6 g total fat per 30 g serving size (total fat 92%) [11], it is possible to calculate the corresponding value for the *trans* fat content as a percentage of total fat; this value would be (0.5 g/27.6 g) × 100 = 1.8% *trans* fat (as a

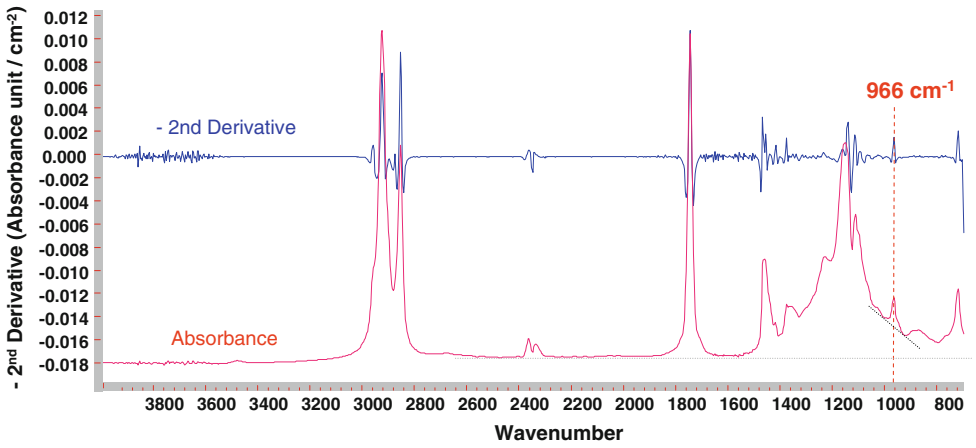

Fig. 1 Negative second-derivative (*top*) and absorption (*bottom*) spectra for a trielaidin in tripalmitin test sample with a *trans* level of 12.58% (as a percentage of total fat). A *vertical line* indicates the position of the unique band due to isolated *trans* double bonds at 966 cm^{-1}. The second-derivative spectrum was multiplied by −1 solely to make the bands point upwards for convenience

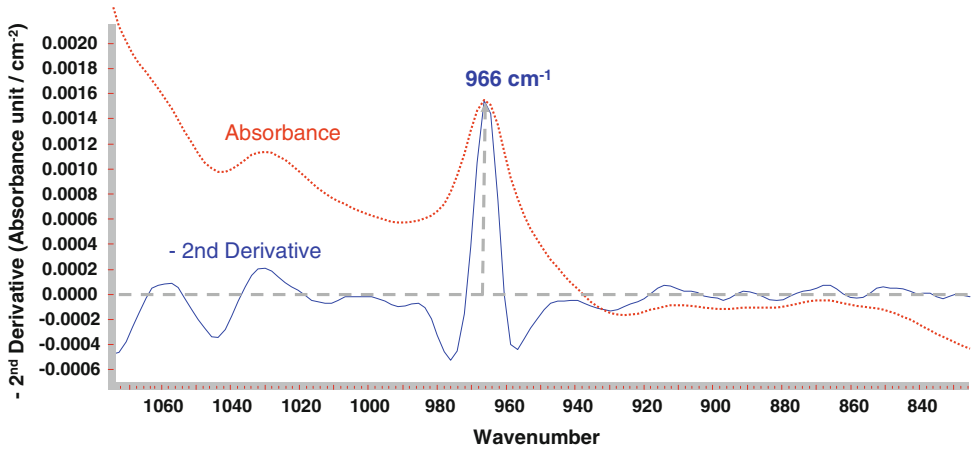

Fig. 2 The region of the spectrum that contains the deformation band for isolated *trans* double bonds at 966 cm^{-1} is expanded for the negative second-derivative (*solid line*) and absorption (*dotted line*) spectrum for a trielaidin in tripalmitin test sample with a *trans* level of 12.58% (as a percentage of total fat). The height of the negative second-derivative band, as indicated by the *vertical arrow*, can be accurately measured from a horizontal baseline. It is noted that several weak bands observed in the same spectral region are also more pronounced in the negative second-derivative spectrum (*narrower* bandwidths) than in the absorption spectrum

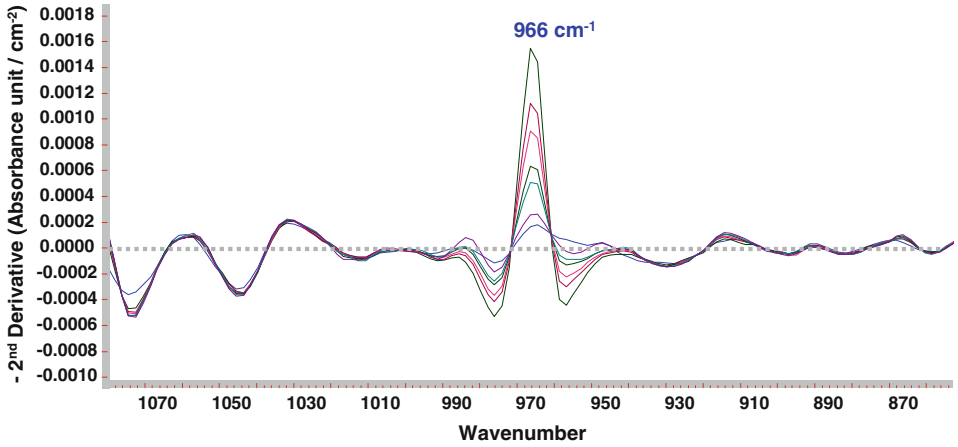

Fig. 3 Expanded spectral region showing the negative second-derivative of the deformation band for isolated *trans* double bonds at 966 cm^{-1} for representative test samples covering approximately the 1–12% range and containing *trans* fat levels determined by IR to be for lard 1.21%, and for Canola oil mixtures 2.21, 4.20, 4.72, 7.35, 9.11, and 12.62%, as a percentage of total fat. The height of the negative second-derivative band can be easily measured from the horizontal baseline (*dotted line*)

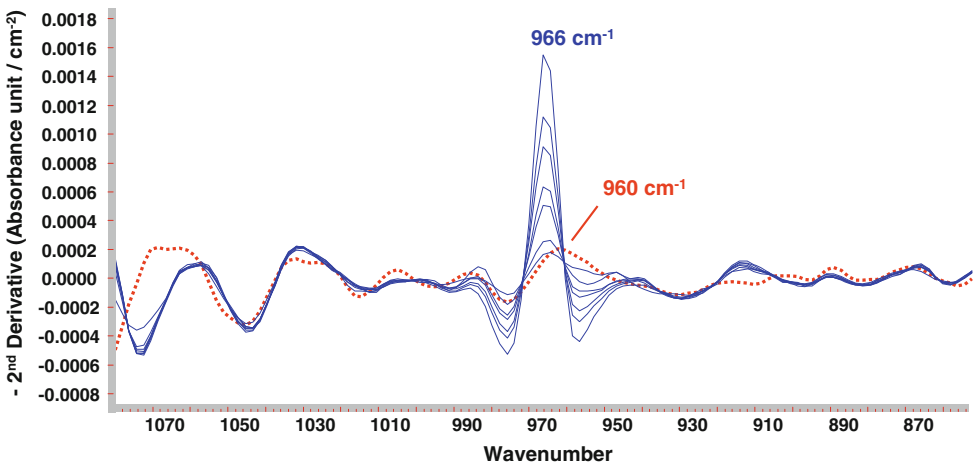

Fig. 4 Expanded spectral region showing the negative second-derivative of the deformation band for isolated *trans* double bonds at 966 cm^{-1} for several test samples containing *trans* fat (*solid blue lines*), and for coconut oil (*dotted red line*) that is high in saturated fat and contains only a trace (approximately 0.1% [7]) of *trans* fat. Coconut oil exhibited a spectral feature at slightly lower wavenumbers, near 960 cm^{-1}, which is easy to misidentify as a band for isolated *trans* double bonds [4]

percentage of total fat). This is the *minimum* level of *trans* fat in a product that had to be measured with confidence in this collaborative study to meet the declaration requirement of zero *trans* fat on the nutrition fact label. For food products containing less total fat, the level of *trans* fat in the total fat of the product would be higher than 1.8% and hence easier to determine. For example, for food products with a low total fat content per serving, typically 3 g per 28 g

serving size (total fat 11%), a *trans* fat level of 0.5 g per serving corresponds to a *trans* fat content of (0.5 g/3 g) × 100 = 17% *trans* fat, as a percentage of total fat. The sensitivity of the newly adopted 2009 ATR-FTIR AOCS Cd 14e-09 official method [12] was adequate and was found to be suitable for the US regulatory compliance monitoring of *trans* fat levels in food products.

Materials and Methods

Materials

Lipid standards were obtained from suppliers such as Nu Check Prep (Elysian, MN, USA) and Sigma (St Louis, MO, USA). Calibration standards were prepared gravimetrically from trielaidin (TE) in tripalmitin (TP) at *trans* fat levels in the range from 0.5 to 20% (as a percentage of total fat). TE calibration standards were prepared by weighing accurately to the nearest 0.0001 g, (0.3−x) g of a saturated TP *trans*-free reference oil, and x g of TE, into a 10 mL vial, where x equals 0.0015, 0.0030, 0.0060, 0.0150, 0.0300, 0.0450, and 0.0600 g, in order to prepare 0.5, 1, 2, 5, 10, 15, and 20% (as a percentage of total fat) *trans* calibration standards, respectively.

Test Samples

A total of ten test samples that covered the range 1–12% *trans* fat (as a percentage of total fat) were quantified [6] in this ATR-FTIR collaborative study. The test samples investigated included two test samples that had been collaboratively studied for validation of AOCS Ce 1h-05 GC official method [7], because means within the range of interest, namely 0.90% (lard) and 11.62% (margarine oil) *trans* fat (as a percentage of total fat), had been reported. In addition, six more test samples were also collaboratively measured by ATR-FTIR (and by GC in a single laboratory) and consisted of mixtures of partially hydrogenated canola oil and canola oil with *trans* levels of approximately 2, 4, 5, 7, 9, and 12%, as a percentage of total fat; canola oil and partially hydrogenated canola oil were obtained from a commercial supplier and prepared from the same lot. The lard and margarine oil test samples were also used as blind duplicates in this ATR-FTIR study. The last six test samples were processed (refined and deodorized) specific mixtures of canola oil and partially hydrogenated canola oil that were acquired commercially [6].

ATR-FTIR Spectroscopy

IR measurements were carried out on infrared spectrometers such as a Varian (Lexington, MA, USA) FTS 7000e IR spectrometer operating under Resolution Pro software in the attenuated total reflection (ATR) mode. The optical bench included a Michelson interferometer with an air bearing moving mirror, a potassium bromide substrate beam splitter, and a mercury cadmium telluride (MCT) detector. The spectrometers used were equipped with a heated internal reflection element made of zinc selenide (ZnSe), diamond, or equivalent with a capacity of approximately 1–10 μL; the equipment must be capable of maintaining the ATR crystal at a constant temperature of 65 ± 1 °C. The cell was warmed to approximately 65 °C in order for test samples, which consisted of neat (not diluted in any solvent) fats and oils, remained melted during analysis. The spectrometer performance met the following criterion: in the absence of a test sample, a 3 min data collection at 4 cm^{-1} resolution must yield between 1,050 and 900 cm^{-1} a peak-to-peak noise level <0.0005 absorbance units (AU) for absorption spectra. To achieve a minimum signal-to-noise ratio (SNR) of 10:1, a high-sensitivity linearized MCT detector operating at liquid nitrogen temperature was recommended when a single-reflection ATR crystal was used. For room temperature deuterium triglycine sulfate (DTGS) detectors, a 3-reflection (or higher) ATR crystal was recommended. FTIR spectra were collected over the wavenumber range 4,000–700 cm^{-1} at a resolution of 4 cm^{-1}. To enhance the signal-to-noise ratio, 256 scans were co-added and signal averaged.

ATR-FTIR Procedure

The conditions used were identical for test samples and calibration standards. Using a disposable pipette, approximately 1–10 μL was transferred without weighing to cover the entire surface of the ATR crystal. The reference background material used was air. The single-beam spectrum to be used as background was collected and saved. The neat test sample was placed (without weighing) on the horizontal ATR crystal, and the single-beam spectrum was collected and saved. The test sample single-beam spectrum was then ratioed against that of the background single-beam spectrum, converted to absorbance, and saved. To enhance spectral features, the negative second derivatives of the absorption spectra were generated [6].

ATR-FTIR *trans* Fat Determination

Calibration standards were measured by ATR-FTIR and the heights of the negative second-derivative absorption bands were recorded. A calibration plot of height versus *trans* level was generated and a regression fit was obtained. Using the slope and intercept of the linear regression equation generated for TE calibration standards, the *trans*

level (as a percentage of total fat) for each fat or oil test sample was calculated by substituting the height of the negative second-derivative *trans* band into the equation: $\%trans = (\text{height} - \text{intercept})/\text{slope}$. Results were recorded to the nearest 0.01%. This equation assumes that *trans* test samples consist of TE. The resulting collaborative study data for unknown test samples were analyzed according to the AOACI Statistical Program 2001 [13] developed by the AOCS Statistics Committee (AOCS International, Gaithersburg, MD, USA).

Gas Chromatography

A Hewlett–Packard Model 5890 Series II GC equipped with a flame-ionization detector, an autosampler (HP Model 7673), and a 100-m CP-Sil 88 fused capillary column (Varian, Mississauga, ON, USA) was used. Hydrogen was used as the carrier gas at a flow rate of 1 mL/min. The *trans* fatty acids were analyzed using two complementary GC temperature programs [14]. Briefly, the two temperature programs were: 45 °C held for 4 min, increased at 13 °C/min to 175 °C and held for 27 min, then increased at 4 °C/min to 215 °C and held for 35 min. The second program was: 45 °C held for 4 min, increased at 13 °C/min to 150 °C and held for 47 min, then increased at 4 °C/min to 215 °C and held for 35 min. Details of *trans* fatty acid identification and determination by GC have been described elsewhere [14].

Results and Discussion

The ATR-FTIR official method Cd 14e-09 [12] was designed to determine in a single measurement the level of total isolated (non-conjugated) *trans* fats in *trans*-monounsaturated or mono-*trans*-polyunsaturated molecules with an isolated, methylene-interrupted, or non-methylene-interrupted double bond(s). The objective of this study was:

1. to overcome the high variability in the precision of measurements of *trans* fats and oils at levels below 5% (as a percentage of total fat) with infrared Official Methods AOCS Cd14d-99 [1] and AOAC 2000.10 [2]; and
2. to meet the US labeling requirements by validating test samples with *trans* levels of approximately 1.8% and higher *trans* fat (as a percentage of total fat).

Therefore, this collaborative study focused on evaluating *trans* fat levels between approximately 1 and 12% *trans* fat (as a percentage of total fat).

In order to improve accuracy and precision a number of experimental precautionary notes were recommended and used in this study. Specifically, it was essential to ensure that the test portion of the fat being analyzed completely covered the horizontal surface of the ATR crystal for the quantitative determination to succeed. In rare cases, after placing a fat test sample on the ATR crystal, the melted or liquid fat may form beads and partially roll off the surface of the ATR crystal. In such cases repeating the measurement is recommended. Optimum cleaning of the ATR crystal was also critical to the success of the determination. It was recommended to clean the crystal without the use of any solvent by thoroughly wiping the horizontal surface of the ATR crystal with low-lint paper. However, to ensure complete removal of a test sample the analyst should also apply the subsequent test portion to be analyzed, and clean the crystal once again. Additionally, the analyst could make an infrared measurement after cleaning the crystal; the absence of a fat spectrum would confirm that the crystal is clean. This last step was particularly useful when a test sample having a relatively low level of *trans* fat was measured after another unknown test sample with a high *trans* fat content. However, for accurate background measurement (in the absence of a test sample), more precautions were required, and the use of a lint-free and glue-free cotton swab with a very small amount of ethanol followed by drying with a cotton swab was recommended. In all cases, a blank ATR-FTIR measurement would confirm the removal of all residues and solvents.

Analysts were requested to perform duplicate measurements of test samples, including the blind duplicate test materials (Table 1). The terms used for the statistical data listed in Tables 2 and 3, namely s(r), repeatability standard deviation; s(R), reproducibility standard deviation; RSD(r), repeatability relative standard deviation; RSD(R), reproducibility relative standard deviation; and HORRAT value (http://www.aoac.org/dietsupp6/Dietary-Supplement-website/HORRAT_SLV.pdf), are given in the AOAC Harmonization Guidelines [13].

Precision

The repeatability Cochran outlier test [13] dictated the removal of the extreme values 2.78 and 2.84 (Table 1) for test sample 1 (with an overall mean of 2.15, Table 2) from laboratories 2 and 5, respectively, because they showed significantly greater variability among duplicate analyses than those reported by other laboratories. Similarly removed on the basis of this test were the extreme value 9.74 for test sample 5 (with an overall mean of 9.14) from laboratory 3 and the extreme value 10.87 for test sample 6 (with an overall mean of 12.55) from laboratory 6. The reproducibility Grubbs' outlier test [13] for removal of laboratories with extreme averages revealed high outlier results, 1.88 and 1.88 (Table 1), from laboratory 2 relative to the overall mean of 1.29 for test sample 8 (Table 2). The

Table 1 Collaborative ATR-FTIR study results

Lab. no.		1	1	2	2	3	3	4	4	5	5	6	6	7	7	8	8	9	9	10	10
Test sample[a,b]																					
Canola mixture	1	2.18	2.15	2.78[c]	2.33	1.78	1.90	1.94	2.06	2.16	2.84[c]	2.14	2.21	2.16	2.18	2.33	2.34	2.31	2.30	2.09	2.09
Canola mixture	2	4.20	3.78	4.13	4.58	4.59	4.07	4.44	4.19	4.27	4.15	4.31	4.20	4.15	4.35	4.30	4.23	4.49	4.49	4.21	4.21
Canola mixture	3	5.09	4.95	5.48	5.48	5.06	5.37	5.06	5.06	4.98	4.82	4.84	4.72	4.96	5.25	5.22	5.17	5.40	5.39	5.19	5.19
Canola mixture	4	7.21	6.62	7.73	7.28	6.93	6.24	7.25	7.38	7.46	7.32	7.51	7.35	7.31	7.43	7.30	7.18	7.58	7.58	7.23	7.24
Canola mixture	5	8.94	9.21	9.08	9.08	9.74[c]	8.84	9.44	9.13	8.82	9.20	9.29	9.11	9.19	9.23	9.10	8.99	9.45	9.44	9.04	9.04
Canola mixture	6	12.62	12.13	10.87[c]	12.22	12.54	11.88	12.56	12.69	12.63	12.45	12.85	12.62	12.84	12.72	12.46	12.17	12.99	12.99	12.56	12.56
Margarine oil	7	11.98	11.71	11.77	12.67	12.54	11.88	12.56	12.38	12.33	12.76	12.58	12.35	12.60	12.18	12.03	11.56	12.66	12.66	12.10	12.10
Lard	8	1.40	1.21	1.88[d]	1.88[d]	1.31	1.47	1.31	1.19	1.43	1.03	1.28	1.21	1.44	1.19	1.58[d]	1.62[d]	1.34	1.33	1.25	1.25
Lard	9	1.26	1.20	1.88[d]	1.88[d]	1.31	1.47	1.31	1.19	1.33	1.81	1.25	1.24	1.32	1.20	1.58	1.24	1.41	1.40	1.29	1.29
Margarine oil	10	12.25	11.70	12.22	13.12	13.01	11.88	12.56	12.25	13.12	12.87	12.57	12.36	12.60	12.13	12.07	11.49	12.60	12.60	12.05	12.05

Total isolated *trans* fat as a percentage of total fat (provided in duplicate)

[a] Test samples 1–6 consist of mixtures of canola oil and partially hydrogenated canola oil

[b] Test samples 9 (lard) and 10 (margarine oil) are blind duplicates of 8 and 7, respectively

[c] Cochran's outlier

[d] Single Grubbs' outlier

same result (1.88 and 1.88) was also obtained from laboratory 2 for test sample 9 (overall mean 1.34, Table 2), which was a blind duplicate of test sample 8, and was similarly removed. The Grubbs' outlier test also led to the removal of laboratory 8 for test sample 8 with extreme averages of 1.58 and 1.62.

Repeatability

All test samples had repeatability relative standard deviation, [RSD(r)], values calculated from within-laboratory data in the range 1.55 to 4.87 (Table 2), except those found for the two blind duplicate test samples 8 and 9 (lard) with overall means of 1.29 and 1.34, respectively, which gave rise to respective RSD(r) values of 10.68 and 11.19. It is noted that a superior RSD(r) value of 7.44 was reported for this same test sample that was collaboratively studied by GC [7] (GC overall mean 0.90, Table 3).

Reproducibility

The reproducibility relative standard deviation, [RSD(R)], values calculated from among-laboratory data fell in the range 2.03–11.58 as the overall mean decreased from approximately 12.5–1.3 (Table 2). By contrast, a significantly higher RSD(R) value of 21.70 was reported by GC [7] (vs. 11.58 by ATR-FTIR) for the common test sample (lard) with an overall GC mean of 0.9 (Table 3).

HORRAT Value

The HORRAT value (http://www.aoac.org/dietsupp6/Dietary-Supplement-web-site/HORRAT_SLV.pdf) [15] is a useful index of method performance with regard to precision of among-laboratories data. It is the ratio of RSD(R) calculated from the data to the predicted reproducibility relative standard deviation, PRSD(R), calculated from the Horwitz formula: $PRSD(R) = 2C^{-0.15}$, where C is defined as concentration expressed as a mass fraction. For example, for a concentration of 100%, C equals 1.0, and PRSD(R) = 2. Based on empirical data developed from over 10,000 interlaboratory studies published over the last century, the HORRAT value, RSD(R)/PRSD(R), was assigned a magnitude of 1.0 with limits of acceptability between 0.5 and 2.0 (http://www.aoac.org/dietsupp6/Dietary-Supplement-web-site/HORRAT_SLV.pdf) [15]. All the ATR-FTIR data in this study yielded HORRAT values within this range (Table 2), except those obtained for the two blind duplicate test samples 8 and 9 (lard), with overall means of 1.29 and 1.34, respectively, which yielded respective HORRAT values of 2.77 and 3.03. However, a significantly greater HORRAT value of 5.340 was reported [7] for GC analysis of this lard test sample with an overall

Table 2 IR collaborative study precision data

Test sample[a,b]	1	2	3	4	5	6	7	8	9	10
No. of laboratories	10	10	10	10	10	10	10	8	9	10
No. of replicates	18	20	20	20	19	19	20	16	18	20
Mean	2.15	4.27	5.13	7.26	9.14	12.55	12.27	1.29	1.34	12.38
s(r)	0.05	0.21	0.11	0.24	0.14	0.22	0.32	0.14	0.15	0.40
s(R)	0.16	0.21	0.22	0.34	0.19	0.29	0.37	0.14	0.16	0.46
RSD(r)	2.17	4.87	2.14	3.26	1.55	1.76	2.57	10.68	11.19	3.21
RSD(R)	7.41	4.87	4.38	4.71	2.03	2.35	2.99	10.68	11.58	3.68
HORRAT	2.08	1.52	1.40	1.59	0.71	0.86	1.09	2.77	3.03	1.34

[a] Test samples 1–6 consist of a mixture of canola oil and partially hydrogenated canola oil

[b] Test samples 9 (lard) and 10 (margarine oil) are blind duplicates of 8 (lard) and 7 (margarine oil), respectively

Table 3 Comparison of IR collaborative study precision data with those collaboratively obtained by GC (Ce 1h-05) for the two common test samples

	Margarine oil			Lard		
	IR	IR	GC[b] Ce 1h-05	IR	IR	GC[b] Ce 1h-05
Test sample[a]	7	10		8	9	
Mean	12.27	12.38	11.62	1.29	1.34	0.90
s(r)	0.32	0.40	0.16	0.14	0.15	0.07
s(R)	0.37	0.46	0.25	0.14	0.16	0.20
RSD(r)	2.57	3.21	1.38	10.68	11.19	7.44
RSD(R)	2.99	3.68	2.18	10.68	11.58	21.70
HORRAT	1.09	1.34	0.71	2.77	3.03	5.34

[a] Test samples 9 (lard) and 10 (margarine oil) are blind duplicates of 8 and 7, respectively

[b] GC official method Ce 1h-05 (reference [7])

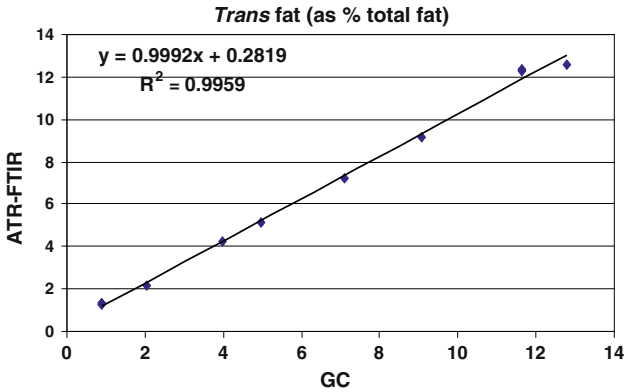

Fig. 5 Plot of the mean *trans* fat values for all the test samples determined collaboratively by ATR-FTIR versus those obtained by GC [7, 14], indicating good agreement between the two techniques

GC mean of 0.9 (Table 3). HORRAT values greater than 2 may indicate several sources of errors including operating below the limit of determination of the method [15].

In addition, the specific mixtures of canola oil and partially hydrogenated canola oil were also analyzed by GC [14] in a single laboratory. For all the test samples investigated, there was good agreement (Fig. 5) between the mean ATR-FTIR values found in this collaborative study and those obtained by GC [7, 14].

In conclusion, no laboratory was found to be an overall outlier laboratory. The ATR-FTIR official method Cd 14e-09 [12] is recommended for determination of total unsaturated fatty acids with isolated *trans* double bonds in oils, fats, partially hydrogenated oils, and in oils and fats isolated from food products containing >0.5 g *trans* fat per serving, which is equivalent to >1.8% *trans* unsaturation (as a percentage of total fat). This method is relatively simple because it requires neither derivatization of the oil or fat test samples nor weighing or quantitative dilution of test samples in any solvent. It is also rapid and requires approximately 5 min for performing the ATR-FTIR measurement and calculation of percentage *trans* levels. This new official method Cd 14e-09 [12] is superior to the most recent IR official method, AOAC 2000.10 [2], which resulted in poor precision for *trans* levels below 5% of total fat.

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