New FTIR Method for the Determination of FFA in Oils

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ABSTRACT: A rapid, practical, and accurate FTIR method for the determination of FFA in edible oils was developed. Analogous to the AOCS titration procedure, the FTIR FFA determination is effected by an acid/base reaction but directly measures the product formed rather than utilizing an end point based on an electrode potential or color change. A suspension of a weak base, potassium phthalimide (K-phthal) in 1-propanol (1-PrOH), is used to convert the FFA present in oils to their carboxylate salts without causing oil saponification, and differential spectroscopy is used to circumvent matrix effects. Samples are first diluted with 1-PrOH, then split, with one-half treated with the K-phthal reagent and the other half with 1-PrOH (blank reagent), their spectra collected, and differential spectra obtained to ratio out the invariant spectral contributions from the oil sample. Quantification of the percentage of FFA in the oil, expressed as %oleic acid, based on measurement of the peak height of the ν (COO^-) absorption of the FFA salt formed, yielded a calibration with an SE of <0.020% FFA over the range of 0–4%. The method was validated by standard addition and the analysis of Smalley check samples, the results indicating that the analytical performance of the FTIR procedure is as good as or better than that of the standard titrimetric procedure. As structured, the FTIR procedure is a primary method, as calibration is not dependent on reference values provided by another method, and has performance criteria that could lead to its consideration as an instrumental AOCS procedure for FFA determination. The FTIR portion of the analysis is automatable, and a system capable of analyzing ~60 samples/h was developed that could be of benefit to laboratories that carry out a large number of FFA analyses per day.

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KEY WORDS: Automated analyses, edible oils, FFA, FTIR, potassium phthalimide, 1-propanol.

FTIR spectroscopy is playing an increasingly important role in the analysis of edible oils by providing simpler and more rapid techniques for determining common oil quality parameters (1). FFA are common TAG hydrolysis products in crude oils and are formed to some extent in refined oils as a result of oxidation or TAG degradation during frying, impairing oil quality and functionality. Chemically, FFA are less stable than TAG and therefore more likely to oxidize and cause rancidity (2). The standard method commonly used for FFA analysis is based on the titration of an oil dissolved in alcohol with a strong base to a phenolphthalein end point (3,4). Although simple, titrimetric methods are tedious, consume substantial amounts of solvent, and can be problematic when dark crude oils are analyzed. The first application of FTIR spectroscopy for FFA analysis was reported by Lanser *et al.* (5) in 1991. In this method, which was developed for crude soybean oil, the FFA content was estimated from the FFA ν (C=O) band at 1710 cm^{-1}. Owing to the overlap of this band with the very strong TAG ester carbonyl absorption at 1746 cm^{-1}, spectral deconvolution over the 2000–1600 cm^{-1} range was used to enhance spectral resolution mathematically. A calibration was derived by spiking oleic acid into soybean oil at levels of 0.1–5% and yielded predictions of the FFA content of soybean oils that matched the values obtained using the AOCS titrimetric method to within ±0.5 percentage points. However, because the FTIR spectra were acquired by simply placing each sample between two KBr windows, without the use of an internal standard, the accuracy of this method was limited by the resulting variability in pathlength. In 1993, Ismail *et al.* (6) investigated two different FTIR approaches to the quantitative determination of FFA in edible oils. The first was based on measuring the carboxylic acid ν (C=O) band at 1711 cm^{-1} in spectra acquired from oil samples applied in their neat form onto an attenuated total reflectance (ATR) crystal. Both calibration and sample spectra were ratioed against the spectrum of an FFA-free oil of the same type as the oil being analyzed to reduce matrix effects. The second approach was an indirect method based on the use of KOH/methanol to extract the FFA present in the oil and convert them to their salts, followed by measurement of the ν (COO^-) absorption band at 1570 cm^{-1} in the spectrum of the methanol phase. This indirect method enhanced the sensitivity of the analysis by concentrating the FFA in a small volume of methanol and by utilizing an absorption band that is in a region free from major oil spectral interferences. However, it had the disadvantage of requiring an additional procedural step, and some saponification of oils by the KOH/methanol reagent added was noted, resulting in overestimation of the FFA content of the original sample. Verleyen *et al.* (7) devised a more rigorous version of the method of Lanser *et al.*, using peak height measurements at 1711 cm^{-1} to develop workable calibrations for a variety of oils, but ultimately concluded that the calibrations were strongly oil dependent. Two other publications have dealt with FFA analysis by FTIR spectroscopy, specifically in relation to palm and olive oil, respectively (8,9), both using a partial-least-squares (PLS) regression to develop

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relationships between spectral changes and results obtained by standard methods. The most sophisticated methodology is that of Cañada *et al.* (10), who developed an automated FTIR-based continuous-flow analysis system capable of analyzing ~40 samples/h using the indirect approach described by Ismail *et al.* (6). However, even within the ~90 s required for analysis in this automated system, some saponification of the oil can occur. In general, the main drawback associated with the direct FTIR methods is their oil dependency, whereas the indirect FTIR methods are limited by the possibility of errors due to saponification caused by the KOH/methanol reagent. Hence, a simple, reliable, and robust FTIR method for FFA analysis is still lacking.

The McGill IR Group recently developed an FTIR-based instrumental method to replace the American Society for Testing and Materials (ASTM) titrimetric procedures for the determination of acid number (AN) in mineral and ester-based lubricating oils (11). These ASTM methods are similar to those traditionally employed for the determination of FFA content in edible oils except that they measure not only carboxylic acids but also a variety of other acidic constituents, organic or inorganic, that accumulate in lubricating oils either as a result of oxidation or as combustion by-products (12). Because the FTIR AN method was specifically designed to meet the requirements of lubricant analysis, it is not directly applicable to edible oils owing to their different spectral characteristics. However, elements of this methodology have been adapted to develop a new method for FFA determination that overcomes the limitations of both the direct and indirect FTIR methods previously developed for FFA analysis. This paper describes the principles of this method as well as their practical implementation and provides an evaluation of its performance by standard addition as well as by employing AOCS Smalley check samples.

EXPERIMENTAL PROCEDURES

Reagents and standard methods. All reagents used were of analytical grade. Potassium phthalimide (K-phthal, 99+%) and hexanoic acid (99%) were obtained from Sigma-Aldrich (St. Louis, MO); 1-propanol (1-PrOH) and isopropanol were purchased from Fisher Scientific Ltd. (Nepean, ON, Canada). All edible oils were obtained locally, and samples were analyzed for FFA using AOCS method Ca 5a-40 (3). Mineral oil (C-171 polyalphaolefin) was obtained from Thermal-Lube (Montréal, QC, Canada) and used for reagent blank determinations. A series of five oils preanalyzed for FFA content were obtained from the AOCS Smalley Check Sample program.

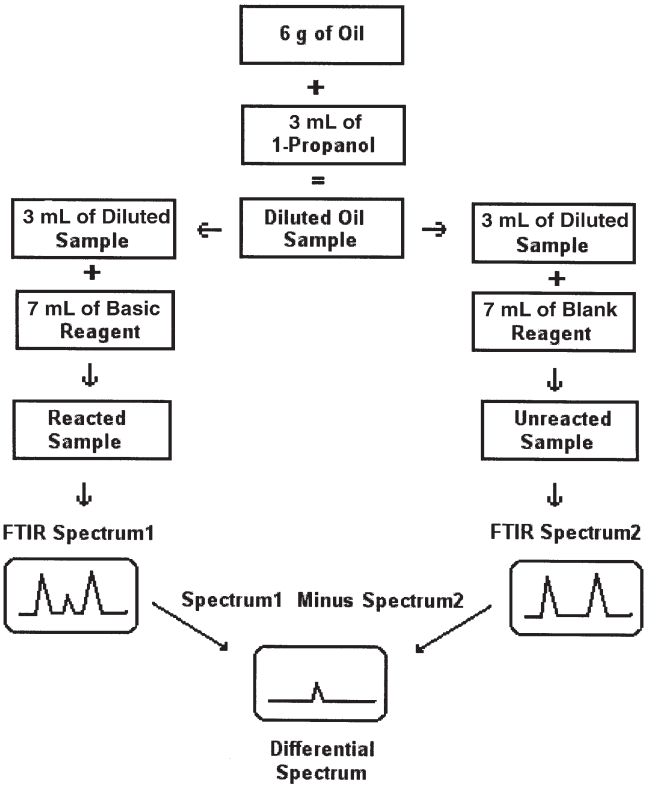
Instrumentation. The instrument used for this study was a Bomem WorkIR spectrometer (Bomem, Québec, QC, Canada) equipped with a deuterated triglycine sulfate detector and purged with dry air from a Balston dryer (Balston, Lexington, MA). Samples were analyzed by aspirating them into a 500-µm CaF_2 transmission flow cell mounted on a sample shuttle (Dwight Analytical, Toronto, ON, Canada). The spectrometer was controlled by an IBM-compatible Pentium 150-MHz PC running under proprietary Windows-based UMPIRE® (Universal Method Platform for InfraRed Evaluation) software (Thermal-Lube, Pointe-Claire, QC, Canada). All spectra were collected by co-adding 16 scans at a resolution of 8 cm^{-1} and a gain of 1.0.

Preparation of calibration standards. A series of eight standards covering the range 0–4% FFA (expressed as the percentage of oleic acid) were prepared by gravimetric addition of hexanoic acid to a refined and deodorized soybean oil. The calibration curve was obtained by linear regression of %oleic acid (%FFA) against the peak heights in the FTIR spectra recorded for the standards by following the sample preparation and analytical protocols described below.

Sample preparation for FTIR analysis. Six grams of the oil sample was mixed with 3 mL of 1-PrOH in a 20-mL vial. Aliquots (3 mL) of the diluted oil were placed in two centrifuge tubes, labeled BR (blank reagent) and RR (reactive reagent), to which were added 7 mL of 1-PrOH and 7 mL of K-phthal/1-PrOH (20 g/L), respectively. All tubes were capped, shaken on a vortex mixer, and then centrifuged for a minimum of 5 min at $5230 \times g$ in a clinical centrifuge. It should be noted that K-phthal is virtually insoluble in 1-PrOH and was dispensed *via* bottle repipette as a fine dispersion that was maintained by continuous and vigorous agitation on a magnetic stirrer.

Analytical protocol. The transmission flow cell was loaded with ~2 mL of the BR sample, and its single-beam spectrum was recorded. After the cell was flushed with isopropanol, ~2 mL of the RR sample was loaded into the cell and its single-beam spectrum was recorded and ratioed against that of its corresponding BR sample to produce a differential spectrum. For quantification of FFA, the peak height of the carboxylate ν (COO^-) band at 1570 cm^{-1} in the differential spectrum was measured relative to a baseline point at 2150 cm^{-1}. A schematic diagram of the sample preparation procedure and analysis is presented in Scheme 1.

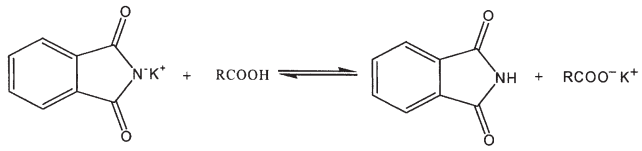
Validation. The FTIR method was validated by standard addition of FFA to soybean and corn oil and by analyzing AOCS Smalley check samples, which included five types of oils (crude coconut, crude corn, crude safflower, cottonseed, and marine oils). For the standard addition experiments, an FFA mixture was prepared by saponifying olive oil with 50% wt/vol KOH followed by titration with 6 M HCl to regenerate the FFA (13), extraction into *n*-hexane, removal of the solvent using a rotary evaporator, and titration of the residue to determine %FFA (expressed as %oleic acid). Soybean and corn oils were then spiked (wt/wt) with six levels of this FFA mixture. These samples and the Smalley check samples were analyzed for their FFA content by the FTIR method as well as by the AOCS titrimetric method. Reproducibility was evaluated as the SD around the mean of triplicate analyses (SD_r). For the standard addition experiments, accuracy was assessed in terms of mean difference (MD_a) and SD of the differences (SDD_a) with respect to the gravimetrically spiked amounts of FFA. In the case of the Smalley check samples, the FTIR results were compared to the results of certified laboratories that had analyzed the samples by the AOCS titrimetric procedure, using the statistical data (mean, minimum, and maximum) provided with the samples.



SCHEME 1

RESULTS AND DISCUSSION

Analytical concepts. As noted, FTIR methods developed to date for FFA analysis have been either matrix dependent or prone to saponification errors. In FTIR methodology developed for AN analysis in lubricants, the weak base K-phthal, a dicarbonyl compound, served as a *signal-transducing reagent* by reacting with all organic and inorganic acids present to form a single product, phthalimide, allowing AN to be determined from the intensity of phthalimide's strong ν (C=O) band at 1729 cm^{-1}, with differential spectroscopy then being used to eliminate the oil matrix effects (12). This method is not directly applicable to edible oils, because the 1729 cm^{-1} phthalimide band is masked by overwhelming absorptions of the ester linkages of the TAG, whereas the second phthalimide ν (CO) band at 1773 cm^{-1} is too weak to measure FFA concentrations of <1%. However, for the analysis of FFA, signal transduction is not required *per se*, only the stoichiometric conversion of FFA to their salts, which can then be quantified directly by measurement of their ν (COO^-) absorption. Moreover, the base used should be too weak to hydrolyze TAG, unlike the KOH employed in previous work (6,10). K-phthal (pK_a = 9.9) was found to meet this criterion, with the additional advantage that 1-PrOH could be used to deliver this reagent into oils, thereby eliminating phase separation, with the FFA salts remaining soluble in the 1-PrOH/oil mixture. The stoichiometric reaction of K-phthal with FFA is presented in Scheme 2:



SCHEME 2

As in lubricant AN methodology, the problem of matrix effects, which may arise in analyzing different oil types or result from the presence of various minor constituents in the samples analyzed, is addressed by utilizing differential spectroscopy. This approach involves splitting the sample into two parts; one part is then treated with K-phthal in 1-PrOH (designated RR), whereas the other portion is treated only with an equivalent amount of 1-PrOH (designated BR) and serves as a reference for the reacted sample. Since the spectral features of the oil are invariant in the spectra of the BR- and RR-treated portions, they are canceled out in the differential spectrum and only the spectral changes associated with the acid/base reaction are left for evaluation (Scheme 1).

Calibration and stability of the reaction. Figure 1 illustrates typical differential spectra obtained when calibration standards (soybean oil spiked with hexanoic acid over a range of 0–4% FFA) were analyzed using the protocol described above. As the amount of hexanoic acid increased in each subsequent standard, the ν (COO^-) signal at 1570 cm^{-1} and that of the phthalimide ν (C=O) band at 1773 cm^{-1} rise concurrently. The loss of the ν (C=O) band of hexanoic acid, which would manifest itself as a strong negative band around 1710 cm^{-1}, is largely lost in the noise (1770–1700 cm^{-1}) resulting from the subtraction of the off-scale bands of the ester linkage of the oil. A mean plot with SD bars for three sets of hexanoic acid calibration standards obtained by measuring the ν (COO^-) band at 1570 cm^{-1} referenced to 2150 cm^{-1} vs. FFA concentration

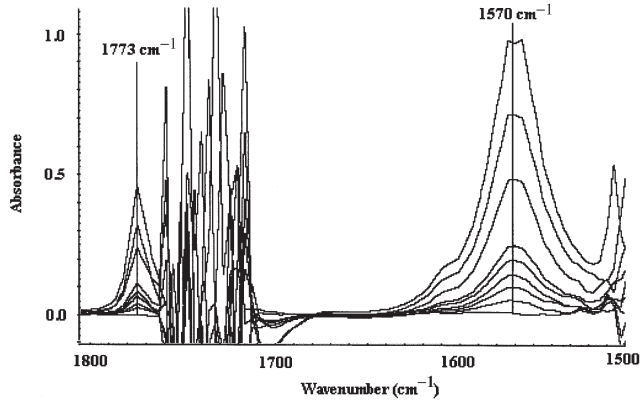


FIG. 1. Differential spectra for soybean oil spiked with hexanoic acid using potassium phthalimide as a reagent to carry out the acid/base reaction. Spectra were recorded in a 500-μm cell at 8 cm^{-1} resolution. The bands at 1773 and 1570 cm^{-1} are due to the phthalimide and hexanoate, respectively, formed in the reaction. The region between 1760 and 1710 cm^{-1} is obscured by noise in the differential spectrum because of the intense oil absorption in this region.

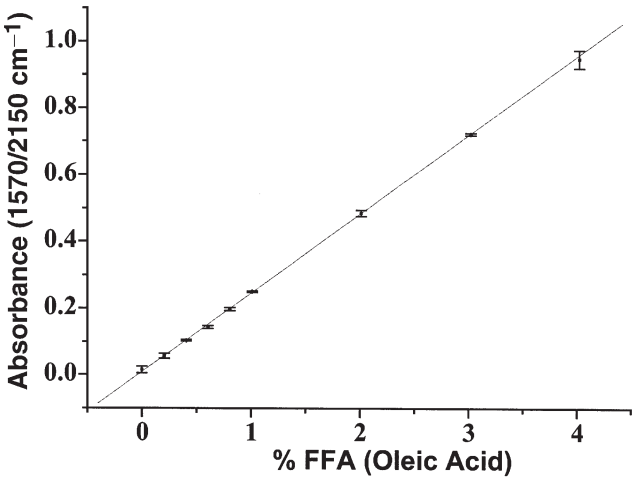


FIG. 2. Composite calibration curve obtained from the differential spectra of three independent sets of hexanoic acid-spiked soybean oil standards of the type illustrated in Figure 1.

expressed as %oleic acid is presented in Figure 2. The overall linear regression equation obtained for the composite calibration was:

$$\%FFA_{(oleic)} = 4.281*A_{(1570/2150)} - 0.0436$$
$$R^2 = 0.9999,\ SD = 0.020 \quad [1]$$

The linearity of the composite calibration plot, with an intercept well within 3σ the regression SD, and the overall SD of ~0.02% FFA indicate that the three individual calibrations that were performed gave highly consistent results. However, calibrations tended to drift over a period of a week, and this was found to be due to changes in the K-phthal reagent, making it necessary to perform a reagent blank correction. Although K-phthal is practically insoluble in 1-PrOH and is delivered as a suspension, small amounts do solubilize slowly over time. Because the solubilized K-phthal has absorptions that overlap with the ν (COO^-) band used to quantify the FFA salts, it can introduce a measurable bias into FFA measurements over time, albeit taking several weeks to develop in a freshly prepared reagent. To account for this reagent background signal, a mineral oil, which does not contain any FFA, is run as a blank to compensate for absorptions contributed by any solubilized K-phthal. The apparent FFA contribution of the blank is subtracted from the values obtained for all oil samples subsequently analyzed to account for any changes in K-phthal concentration.

Duplicate analyses of calibration standards were conducted 24 h apart to confirm that K-phthal does not attack TAG. Based on the MD_r and SDD_r (SD of the difference for reproducibility) of 0.17 and 0.024%, respectively, it was concluded that no significant saponification took place over that time period. This finding was corroborated by similar studies with samples of various refined oils.

Evaluation and validation. To establish the efficacy of the methodology developed, standard addition experiments were carried out by adding a pre-prepared mixture of FFA obtained from olive oil to both refined corn and soybean oils, which were then analyzed in triplicate by the FTIR and the titrimetric AOCS method. The data obtained using these two methods are summarized in Table 1, with the accuracy of both methods being assessed by using the gravimetrically added amounts of FFA as the reference values.

In terms of overall reproducibility, the FTIR method had a mean SD_r of 0.029%, slightly better than that of the AOCS procedure (0.038%). In terms of overall accuracy relative to gravimetric standard addition, both methods have small positive biases, slightly greater than the overall SD_r, indicating that both the soybean and corn oils contained traces of FFA prior to standard addition. For each method, the value of SDD_a, which is a

TABLE 1
Results of Triplicate Analyses of Oils Spiked with Various Amounts of an FFA Mixture by the AOCS Titrimetric and FTIR Methods[a]

	FFA spiked	AOCS method		FTIR method	
Oil	(% wt/wt)[b]	Mean	SD	Mean	SD
Soybean	0.000	0.028	0.003	0.015	0.032
	0.501	0.524	0.015	0.526	0.019
	1.002	1.063	0.034	1.042	0.035
	1.508	1.543	0.061	1.549	0.031
	2.003	2.033	0.029	2.056	0.032
	2.490	2.531	0.055	2.505	0.037
Corn	0.000	0.057	0.011	0.066	0.020
	0.500	0.546	0.009	0.573	0.027
	0.999	1.120	0.053	1.038	0.023
	1.475	1.573	0.068	1.512	0.005
	2.002	2.094	0.076	1.991	0.088
	2.502	2.578	0.051	2.548	0.002
Mean SD_r		0.038		0.029	
MD_a		0.059		0.036	
SDD_a		0.030		0.022	

[a]Abbreviations: SD_r, SD for reproducibility; MD_a, mean difference for accuracy; SDD_a, SD of the differences for accuracy.
[b]Expressed as %oleic acid.

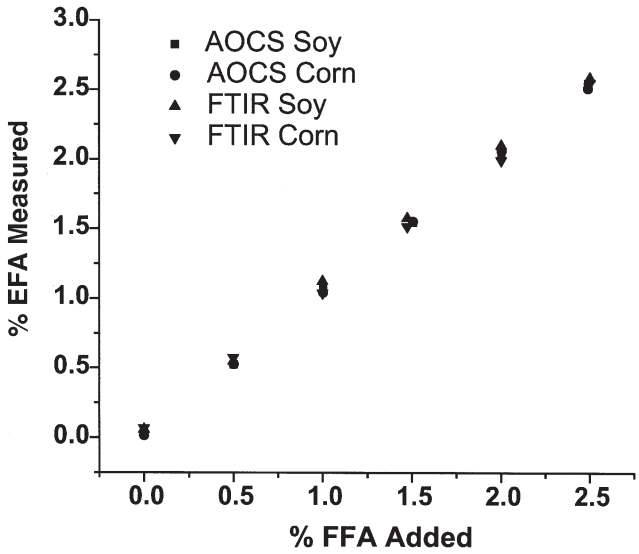


FIG. 3. Graphical comparison of results obtained by the AOCS and FTIR methods for the oils that were subjected to standard addition of FFA mixture, relative to the gravimetrical data.

measure of the variability around the MD_a, is of the same order of magnitude as the SD_r value, with the FTIR method again performing slightly better. Figure 3 presents a composite plot of all the FFA results obtained for both oils by both methods against the reference gravimetric data. The regression equation for the composite data illustrated in Figure 3 is

$$FAA_{(IR/titration)} = 0.99996\ FFA_{(standard\ addition)} + 0.047$$
$$SD = 0.030 \quad r = 0.9994 \qquad [2]$$

The plot and regression equation illustrate the excellent concurrence between the titrimetric and FTIR methods as well as their comparable ability to track the amounts of FFA added gravimetrically to the two oils.

To further validate the performance of the FTIR method, five AOCS Smalley check samples were analyzed in triplicate. The results obtained relative to the analytical data provided with these oil samples are presented in Table 2. In general, there is excellent concurrence between the FTIR mean and Smalley mean FFA values except for crude coconut oil. Analyses of the Smalley samples in our laboratory by the AOCS method were also in line with the Smalley means, except again for crude coconut oil, which produced a value of 0.312 ± 0.005, very much in line with the FTIR result obtained, suggesting that the FFA content of this sample had changed in the time that had elapsed since its analysis in laboratories participating in the Smalley check sample program. Considering the results for the Smalley check samples as well as those obtained by standard addition, it is evident that the FTIR method is capable of producing accurate and reproducible FFA data independent of oil type and that it appears to be a valid alternative to the AOCS titrimetric procedure.

The FTIR methodology for FFA analysis described above combines the respective advantages of the direct and indirect approaches previously described in the literature and overcomes their limitations. As in other indirect approaches, enhanced sensitivity by comparison with direct measurement of the FFA ν (CO) band is achieved by reacting the FFA with a base and measuring the ν (COO^-) band of the salt formed. However, a key difference is the use of a K-phthal suspension in 1-PrOH as a reagent instead of the KOH/methanol reagent previously used (6,10), as its weakly basic properties provide a means of avoiding saponification. Furthermore, the extraction step previously required in the indirect FFA methods is eliminated because no phase separation occurs and the FFA salts remain soluble in the 1-PrOH/oil mixture. The use of differential spectroscopy provides a means of minimizing matrix effects by canceling out the spectral contributions of the oil and contaminants therein, and spectral interferences arising from the slight solubilization of K-phthal in a non-freshly prepared reagent are compensated for by performing a reagent blank correction. These combined procedural elements allow a calibration to be developed by utilizing gravimetrically prepared standards of hexanoic acid in oil. The net result is that the FTIR method developed is a *primary method*, independent of other methods for calibration. It is also rapid and simple to execute, and the FTIR portion of the analysis has been automated by integrating the spectrometer with an autosampler (Thermal-Lube Inc., Pointe-Claire, QC, Canada), allowing for the analysis of up to 60 paired samples per hour. Given that measurements at only two wavelengths are needed for this analytical procedure, a simple dual-wavelength filter-based IR instrument could also be used. With these hallmarks of a sound general method, this new methodology could serve as the basis for an AOCS instrumental method for the analysis of FFA in edible oils.

TABLE 2
Results of Triplicate Analysis of Smalley Check Samples by the AOCS Method and the FTIR Method[a]

	AOCS method					FTIR method	
	Max	Min	Mean	SD	n^b	Mean	SD
Crude safflower oil	0.500	0.330	0.457	0.036	18	0.467	0.057
Cottonseed oil	0.180	0.040	0.130	0.053	8	0.156	0.058
Crude coconut oil	0.200	0.080	0.136	0.029	20	0.297	0.014
Crude corn oil	1.720	1.120	1.419	0.124	19	1.431	0.019
Marine oil	1.970	1.800	1.864	0.044	12	1.810	0.024

[a]Results expressed as the percentage of oleic acid (wt/wt).
[b]*n* = Number of laboratories that reported results for each sample.

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