# Determination of Trace Amounts of Fatty Acids in Edible Oils by Capillary Gas–Liquid Chromatography

**M. Buchgraber[1], F. Ulberth[1,*], and E. Anklam**

European Commission, DG Joint Research Centre, Institute for Health and Consumer Protection, Food Products and Consumer Goods Unit, TP 260, 21020 Ispra (VA), Italy

**ABSTRACT:** The effect of using different gas–liquid chromatography (GLC) hardware to quantify low concentrations of fatty acids was studied. A fused-silica capillary column was operated in two different chromatographs (A and B) that were interfaced to three different chromatographic data systems to process the flame-ionization detector signals (systems A, B1, and B2). A test routine was developed that allowed the proper selection of peak processing parameters for the automatic recognition and integration of fatty acids occurring at trace levels. However, agreement of analytical results between the three analytical systems was not satisfactory; components at concentrations <0.10 g/100 g could not be quantified with high reliability, although the same capillary column and identical sample solutions were used (quasi-repeatability conditions). Even for major fatty acids, deviations up to 1.0 g/100 g were noted, which could only be attributed to the use of different GLC hardware. Attention should be paid to these technical restrictions when formulating product specifications based on fatty acid profile parameters.

Paper no. J9290 in *JAOCS 77*, 653–657 (June 2000).

**KEY WORDS:** Chromatographic data systems, fatty acid methyl esters, gas–liquid chromatography, peak quantification, peak recognition, and *trans* fatty acids.

National and international standardization bodies have issued a large collection of standard methods for the determination of the fatty acid (FA) profile as fatty acid methyl esters (FAME) by gas–liquid chromatography (GLC). Most of these methods (1–3) are based on the $BF_3$/methanol procedure (4), and the majority of standards are designed for use with packed columns. Moreover, in many cases only the gross FA composition of oils is of interest to verify purity and functional properties of edible oils and fats. With the advent of fused-silica technology and improved manufacturing techniques, capillary columns have become very popular and have tended to supersede packed column technology in FAME analysis. However, the already existing standards for FA profiling remain mostly unchanged, although some newer official methods make explicit use of capillary columns (1,5).

Interest in trace levels of FA recently has been spurred by the controversial discussion of health effects of certain FA, e.g., *trans* fatty acids (TFA) (6). With modern, optimized edible oil processing technology it is possible to lower TFA levels in spreads and shortenings to <1% (7). However, TFA levels have to be monitored by appropriate methods of analysis because of consumer concern or legal requirements. In the European Union, for example, the content of total *trans* monoenes has to be lower than 0.03% and the sum of *trans* linoleic plus *trans* linolenic isomers must be lower than 0.03% in virgin olive oil (5). Several official methods describe the technical layout for TFA analysis by capillary GLC, and performance characteristics of these methods have been published (1,5). However, in most instances hardware-related parameters that would enable the detection and quantification of trace levels of certain FA are not specified. A project has been started in the European Union that is targeted at producing oily reference materials with a certified TFA content, including a physically refined oil with a total TFA content of about 1%. During method optimization the need to standardize the protocol for the detection and quantification of low TFA levels was clearly recognized.

In this communication we report the optimization strategy we followed to detect and quantify FAME at the 0.01% level. The procedure proposed can be applied to the quantification of any FAME occurring at trace levels; TFA were only selected to serve as an example. Furthermore, we compared GLC equipment differing in technical sophistication with respect to system suitability.

## MATERIALS AND METHODS

*Materials*. Physically refined soybean oil and rapeseed oil were obtained from Vandemoortele (Izeghem, Belgium); palm oil and a partially hydrogenated sunflower seed oil, from Unilever (Vienna, Austria). Samples of virgin olive oil were bought in a local supermarket. Standard FAME for identification purposes were from Sigma (St. Louis, MO). Solvents used were of AR grade and either from Sigma or from Carlo Erba (Milan, Italy); sodium methylate in methanol (0.5 mol/L) was from Fluka (Buchs, Switzerland).

*GLC*. Instrument A consisted of an HP 6890 Plus GC

[1]Present address: Department of Dairy Research and Bacteriology, University of Agricultural Sciences, Gregor Mendel-Str. 33, A-1180 Vienna, Austria.
*To whom correspondence should be addressed.
E-mail: h610pb@edv7.boku.ac.at

equipped with an HP 7683 autoinjector in combination with the ChemStation software Version A.06.01 (Hewlett-Packard, Cernusco, Italy). A 50 m × 0.25 mm i.d. CP Sil-88 fused-silica column (Chrompack, Middelburg, The Netherlands) was used for FAME separation. The column was operated isothermally at 178°C; injector and flame-ionization detector (FID) temperatures were set to 250°C. Helium at 1.3 bar was the carrier gas. The split ratio was varied between 1:25 and 1:100, and the autoinjector was set to deliver 1 μL samples.

Instrument B was a Carlo Erba Vega 6000 GC (ThermoQuest, Milan, Italy). The same chromatographic conditions were used as for instrument A except that the head pressure of the carrier gas was 1.0 bar, and the column temperature 180°C. Samples were injected manually by the hot-needle technique using a 10 μL syringe. The FID signal was processed by either the Turbochrom-3 (Perkin Elmer, Norwalk, CT) (instrument B1) or by the ChromCard (ThermoQuest) (instrument B2) chromatography data system.

All analog/digital (A/D) converters employed in the chromatographic data systems (CDS) were set to sample 20 data points.

Quantification was done by area normalization, and unity response factors were used throughout. Signal-to-noise (S/N) ratios were determined graphically. Peaks were identified by retention time matching to authentic standards. *Trans* isomers of FAME were identified by reference to elaidinized linseed oil, prepared as described by Grandgirard *et al.* (8).

*Methods.* Sodium methylate-catalyzed transesterification of oils was used to form FAME (9).

## RESULTS AND DISCUSSION

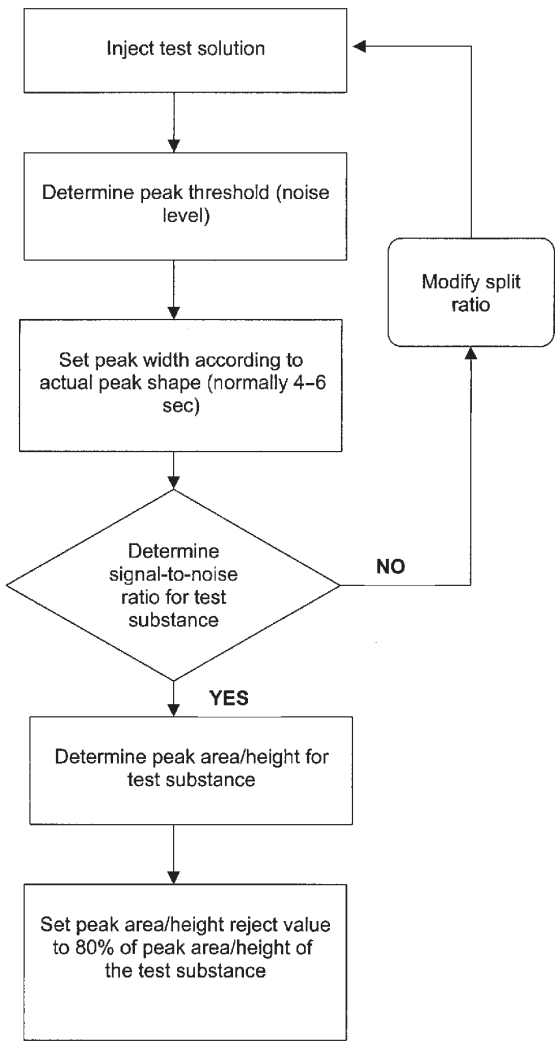
Standard methods, e.g., AOCS Official Method Ce 1c-89 (1), provide the user with detailed guidance regarding the setup of GLC hardware and derivative formation, but a procedure to tune the sensitivity of the analysis in order to meet certain requirements demanded by the analytical task is not always given. In an intercomparison of methods, we observed that in the case of physically refined oils some laboratories reported the amount of *trans* monoenes as "traces," while others were more exact and reported numerical values for them (10).

Sensitivity of GLC analysis is, in general, governed by the design of the detector, the sample amount reaching the detector, and the algorithm that is used for signal processing by the CDS. Since the design of the GLC hardware is fixed, the remaining optimization variables are (i) the concentration of the sample solution, (ii) the amount injected, (iii) the split ratio, and (iv) the settings of the CDS controlling slope sensitivity, peak width, and the area and/or height reject value. The sample amount (factors i-iii) can easily be described. However, it is not possible to describe CDS settings in general terms because they depend heavily on the brand of instrumentation (computer program) used.

As we were interested in the reliable detection and quantification of trace levels of TFA, we defined as a limit of quantitation 0.01 g/100 g total FAME at an S/N ratio of at least 3:1. Peaks exceeding this threshold had to be included in the area normalization procedure. The standard method used in our laboratory to transesterify lipids (8) resulted in a concentration of 12 mg FAME/mL. Consequently, a solution containing 1.2 μg/mL of a particular FAME, which is the concentration corresponding to 0.01% of the total FAME amount, should upon split-injection give a peak with an S/N ratio of at least 3:1 to comply with the stated performance criterion. Oleic acid methyl ester (*cis*9-18:1) was arbitrarily chosen as a model compound, although any other high-purity FAME may be used instead.

The analyst is not required to follow a prescribed procedure but should select proper conditions to fulfill the performance criterion. This should guarantee enough flexibility to accommodate different types of equipment and differences resulting from sample preparation. To guide the operator in choosing appropriate instrument parameters, the flow scheme depicted in Scheme 1 was set up.

Injecting the test solution containing 1.2 μg *cis*9-18:1/mL produced an S/N ratio of the test substance of 3.4:1 and 3.7:1 by using chromatographic systems B1 and B2, respectively.




**SCHEME 1**

**TABLE 1**
**Fatty Acid Profile (g FAME/100 g of total FAME) of Physically Refined Oils[a]**

| | Soybean oil | | | Rapeseed oil | | |
|---|---|---|---|---|---|---|
| FAME | A | B1 | B2 | A | B1 | B2 |
| 14:0 | 0.08 (0.09) | 0.07 (0.07) | 0.07 (0.07) | 0.05 (0.05) | 0.05 (0.05) | 0.05 (0.05) |
| 16:0 | 10.52 (10.49) | 10.98 (10.97) | 10.89 (10.87) | 4.77 (4.76) | 4.86 (4.87) | 4.97 (4.96) |
| 16:1 | 0.09 (0.09) | 0.14 (0.14) | 0.11 (0.11) | 0.22 (0.22) | 0.25 (0.25) | 0.23 (0.23) |
| 17:0 | 0.11 (0.11) | 0.11 (0.11) | 0.10 (0.10) | 0.06 (0.06) | 0.08 (0.08) | 0.04 (0.04) |
| 18:0 | 3.91 (3.90) | 3.76 (3.76) | 3.76 (3.74) | 1.78 (1.78) | 1.61 (1.61) | 1.65 (1.65) |
| 18:1*t* | 0.03 (0.03) | 0.00 (0.02) | 0.03 (0.03) | 0.05 (0.05) | 0.00 (0.00) | 0.03 (0.03) |
| 18:1*c* | 23.86 (23.78) | 23.91 (23.89) | 23.73 (23.70) | 59.30 (59.14) | 60.04 (59.87) | 60.30 (60.22) |
| 18:2*t* | 0.76 (0.78) | 0.43 (0.39) | 0.55 (0.58) | 0.14 (0.15) | 0.13 (0.14) | 0.14 (0.14) |
| 18:2*c* | 52.41 (52.26) | 52.83 (52.82) | 53.12 (53.02) | 21.36 (21.30) | 21.23 (21.29) | 21.24 (21.20) |
| 20:0 | 0.36 (0.36) | 0.33 (0.33) | 0.33 (0.33) | 0.59 (0.59) | 0.53 (0.53) | 0.47 (0.47) |
| 18:3*t* | 0.58 (0.57) | 0.99 (0.99) | 0.52 (0.55) | 0.34 (0.36) | 0.35 (0.35) | 0.30 (0.32) |
| 20:1 | 0.71 (0.71) | 0.19 (0.19) | 0.64 (0.64) | 1.63 (1.63) | 1.40 (1.40) | 1.33 (1.33) |
| 18:3*c* | 5.76 (5.74) | 5.58 (5.57) | 5.54 (5.53) | 8.88 (8.85) | 8.68 (8.70) | 8.59 (8.57) |
| 21:0 | 0.00 (0.03) | 0.04 (0.04) | 0.03 (0.03) | 0.08 (0.08) | 0.07 (0.07) | 0.05 (0.05) |
| 22:0 | 0.46 (0.45) | 0.41 (0.41) | 0.40 (0.40) | 0.34 (0.34) | 0.31 (0.31) | 0.22 (0.22) |
| Area identified (%) | 99.64 (99.41) | 99.77 (99.70) | 99.81 (99.69) | 99.58 (99.35) | 99.59 (99.52) | 99.61 (99.48) |

[a]Mean value of three replicates for the soybean oil and mean value of two replicates for the rapeseed oil. Values in parentheses are those obtained by setting the area/height reject value of the chromatographic data system (CDS) to zero. See text for description of chromatographic systems A, $B_1$, and $B_2$. FAME, fatty acid methyl esters.

The split ratio had to be adjusted to 1:25 to give these values. In contrast, system A was more sensitive and was therefore tuned to a split ratio of 1:100 to produce a similar S/N ratio, (4.2:1). Applying the optimization scheme given in Scheme 1 allowed the setup of suitable integration parameters for the chromatographic systems A and B1. However, when applied to system B2 the outlined procedure failed to recognize the *cis* 9-18:1 peak of the test solution. Even when using a feature found in most CDS, the "auto peak parameter" or "auto integrate" function, which should automatically select the most suitable parameters for peak detection, system B2 failed to recognize the peak of the test substance. Only by trial and error was a suitable combination of peak width and peak threshold parameters found that enabled a proper integration of the test substance.

Setting the peak area/height parameter to zero did not distort the FA profile by integrating noise, but allowed an even more sensitive automatic detection and area estimation of very low amounts of TFA, regardless of the CDS used (Tables 1 and 2). Therefore, we recommend setting the area/height reject value of the CDS to zero and including all peaks attributable to FAME in the estimation of the total peak area. A peak should be excluded only if it is also seen in the chromatogram of a blank run. Setting the reject value to zero increased the total number of integrated peaks—the number of peaks was approximately doubled in most cases—but did not affect the area-% values of identified FAME (Tables 1 and 2, values in brackets).

We were not able to quantify FAME occurring at levels <0.10 g/100 g of total FAME with good reliability. CDS B1

**TABLE 2**
**Fatty Acid Profile (g FAME/100 g of total FAME) of Virgin Olive Oil[a]**

| | Olive oil 1 | | | Olive oil 2 | | |
|---|---|---|---|---|---|---|
| FAME | A | B1 | B2 | A | B1 | B2 |
| 14:0 | 0.00 (0.01) | 0.00 (0.01) | 0.00 (0.01) | 0.00 (0.01) | 0.00 (0.01) | 0.00 (0.00) |
| 16:0 | 9.57 (9.55) | 9.49 (9.48) | 9.49 (9.47) | 12.42 (12.39) | 12.62 (12.61) | 12.57 (12.55) |
| 16:1 | 0.64 (0.64) | 0.58 (0.58) | 0.58 (0.57) | 0.85 (0.85) | 0.83 (0.83) | 0.82 (0.81) |
| 17:0 | 0.06 (0.06) | 0.08 (0.08) | 0.08 (0.08) | 0.08 (0.08) | 0.08 (0.08) | 0.08 (0.08) |
| 18:0 | 3.05 (3.05) | 2.76 (2.76) | 2.76 (2.76) | 2.21 (2.20) | 2.02 (2.02) | 2.02 (2.01) |
| 18:1*t* | 0.04 (0.04) | 0.00 (0.00) | 0.00 (0.00) | 0.03 (0.03) | 0.04 (0.04) | 0.04 (0.04) |
| 18:1*c* | 79.73 (79.61) | 80.67 (80.65) | 80.74 (80.65) | 73.72 (73.59) | 74.35 (74.29) | 74.40 (74.30) |
| 18:2*t* | 0.00 (0.01) | 0.00 (0.00) | 0.00 (0.01) | 0.00 (0.02) | 0.00 (0.00) | 0.00 (0.01) |
| 18:2*c* | 4.88 (4.87) | 4.63 (4.63) | 4.64 (4.63) | 8.75 (8.73) | 8.38 (8.38) | 8.39 (8.37) |
| 20:0 | 0.47 (0.47) | 0.44 (0.44) | 0.44 (0.44) | 0.40 (0.40) | 0.35 (0.35) | 0.35 (0.35) |
| 18:3*t* | 0.00 (0.04) | 0.05 (0.05) | 0.05 (0.09) | 0.00 (0.03) | 0.00 (0.01) | 0.00 (0.02) |
| 20:1 | 0.31 (0.32) | 0.29 (0.29) | 0.30 (0.30) | 0.34 (0.34) | 0.28 (0.28) | 0.30 (0.30) |
| 18:3*c* | 0.63 (0.63) | 0.58 (0.58) | 0.58 (0.58) | 0.66 (0.66) | 0.58 (0.58) | 0.60 (0.60) |
| 21:0 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) |
| 22:0 | 0.14 (0.14) | 0.14 (0.14) | 0.13 (0.13) | 0.11 (0.11) | 0.10 (0.10) | 0.10 (0.09) |
| Area identified (%) | 99.52 (99.44) | 99.71 (99.69) | 99.78 (99.72) | 99.57 (99.45) | 99.53 (99.48) | 99.54 (99.43) |

[a]Mean value of two replicates. Values in parentheses are those obtained by setting the area/height reject value of the CDS to zero. See Table 1 for abbreviations.

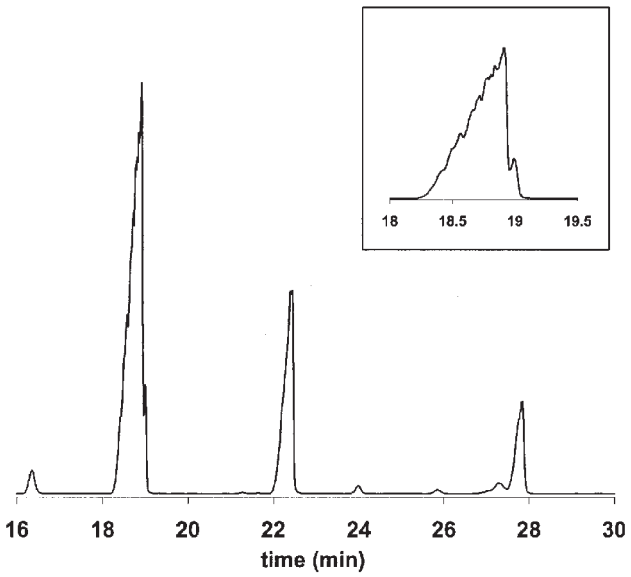



**FIG. 1.** Separation of rapeseed oil fatty acid methyl esters by a 50 m × 0.25 mm CP Sil-88 capillary column (split ratio of 1:25) by chromatographic system B. Inset shows an enlarged view of the *cis*9-C18:1 peak.

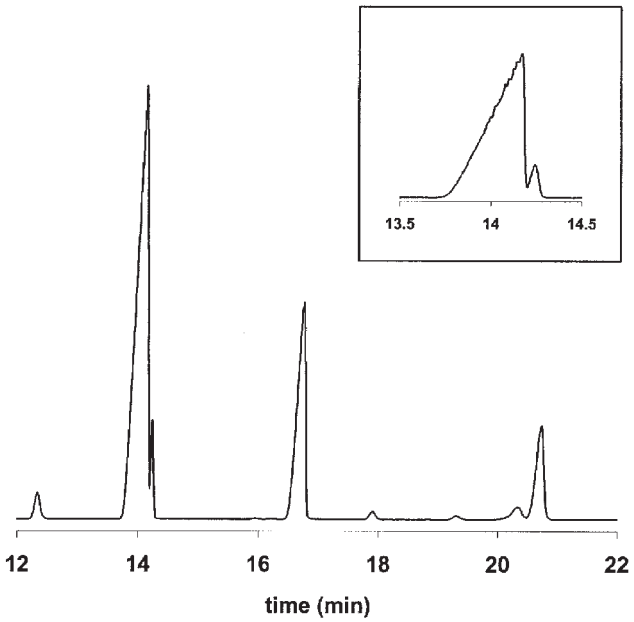



**FIG. 2.** Separation of rapeseed oil fatty acid methyl esters by a 50 m × 0.25 mm CP Sil-88 capillary column (split ratio of 1:25) by chromatographic system A. Inset shows an enlarged view of the *cis*9-C18:1 peak.

failed to quantify *trans* C18:1 isomers in physically refined soybean and rapeseed oils (Table 1), although CDS B1 and B2 processed in parallel the FID output from chromatograph B. Setting the area reject value to zero allowed a proper integration of the *trans* C18:1 isomers in soybean oil but not in rapeseed oil.

Limiting the content of TFA to <0.03 g/100 g of total FAME for high-quality olive oil (virgin oil) is not feasible, given the fact that at such low concentrations the results are heavily dependent on the analytical instrumentation used. This assumption was substantiated by analyzing identical samples of olive oil on two different chromatographs. While olive oil 1 was found using chromatographic system A to be beyond the limit for total *trans* C18:1 isomers, it was within the limit using system B, although the same capillary column and the same sample solution were used for the analyses. The reverse was found for olive oil 2.

Reproducibility, i.e., between-lab variation, would be worse, since other sources of error would add to the variation already found under quasi-repeatability conditions (same lab, operator, reagents, etc.). Even for major FAME, considerable variation was noticed owing to the use of different chromatographic hardware. A difference of 1.00 g of the *cis* C18:1 content of rapeseed oil was found between systems A and B2 (Table 1). Differences of similar magnitude were observed for other major FAME in the other samples analyzed. Moreover, not only different chromatographs but also different CDS used in parallel produced deviating results; differences up to 0.30 g were found (soybean oil and rapeseed oil).

If both the quantification of minor components and resolution of geometrical FAME isomers is required, the narrow-bore capillary columns employed are overloaded as can be seen by the "fronting" of the peak shape of major FAME (Figs. 1 and 2). Manual injection by the hot-needle technique (systems B1 and B2) led to rugged peak shapes and to a segregation of these poorly defined peaks by the peak processing algorithm of the CDS (Fig. 1). Ultrafast sample injection by an automatic sampler (system A) narrowed the starting band-width of the sample plug and thus improved peak shape considerably (Fig. 2). However, overloading the column sacrificed resolution. This resulted in an underestimation of the total *trans* 18:3 isomer content of physically refined soybean oil, where the *trans*9, *cis*12, *cis*15-18:3 isomer partially overlapped with *cis*11-20:1 (Table 1). A near-baseline separation was possible, though, by using a 100 m × 0.25 mm CP Sil-88 capillary column, giving a total *trans* 18:3 isomer content of 1.02 g/100 g and a 20:1 content of 0.23 g/100 g on chromatographic system A.

This procedure to select peak processing parameters is based on an objective criterion and is thus superior to subjective manual evaluation of the chromatogram when measuring trace amounts of FAME. Consequently, it will assist in setting up appropriate quality assurance measures, which are necessary to document the proper operation of the analytical system. The analytical precision attainable in a practical situation should be borne in mind when product specifications derived from the FA spectrum, especially minor compounds, have to be established. Otherwise, the inherent variation of the method may render these specifications obsolete.

## ACKNOWLEDGMENTS

This work was funded by the Standards, Measurements and Testing Programme of the European Commission, contract no. SMT4-CT97-2144.

## REFERENCES


1. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, Fatty Acid Composition by GLC, *cis*, *cis* and *trans* Isomers, American Oil Chemists' Society, Champaign, 1992, AOCS Official Method Ce 1c-89.
2. *Official Methods of Analysis of AOAC International*, Methyl Esters of Fatty Acids in Oils and Fats, Gas Chromatographic Method, 16th edn., AOAC International, Arlington, 1995, AOAC Official Method 963.22.
3. *Standard Methods for the Analysis of Oils, Fats and Derivatives*, Gas–Liquid Chromatography of Fatty Acid Methyl Esters, 7th edn., International Union of Pure and Applied Chemistry, Oxford, 1987, Method 2.302.
4. Christie, W.W., Preparation of Ester Derivatives of Fatty Acids for Chromatographic Analysis, in *Advances in Lipid Methodology—Two*, The Oily Press, Dundee, 1993, pp. 69–111.
5. Commission Regulation (EEC) No. 183/93 of 29 January 1993 Amending Regulation (EEC) No. 2568/91 on the Characteristics of Olive Oil and Olive-Residue Oil and on the Relevant Methods of Analysis, *Official Journal L 022*, 30/01/1993, pp. 58–68.
6. Mensink, R.P., and P.L. Katan, Lipoprotein Metabolism and *Trans* Fatty Acids, in Trans *Fatty Acids in Human Nutrition*, edited by J.L. Sébédio, and W.W. Christie, The Oily Press, Dundee, 1998, pp. 217–234.
7. Ackman, R.G., and T.K. Mag, *Trans* Fatty Acids and the Potential for Less in Technical Products, in *Ibid.*, pp. 35–58.
8. Grandgirard, A., F. Julliard, J. Prevos, and J.L. Sébédio, Preparation of Geometrical Isomers of Linolenic Acid, *J. Am. Oil Chem. Soc. 64*:1434–1439 (1987).
9. Bannon, C.D., G.J. Breen, J.D. Craske, N. Tr. Hai, N.L. Harper, and K.L. O'Rourke, Analysis of Fatty Acid Methyl Esters with High Accuracy. III. Literature Review of and Investigation into the Development of Rapid Procedures for the Methoxide-Catalyzed Methanolysis of Fats and Oils, *J. Chromatogr. 247*:71–89 (1982).
10. Ulberth, F., M. Buchgraber, and A. Boenke, Intercomparison of Methods for the Determination of *Trans* Fatty Acids in Edible Oils and Fats—Results of the 1st Intercomparison Study, *Report EUR 18955 EN*, European Commission, Luxembourg, 1999.


[Received June 23, 1999; accepted February 13, 2000]