JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

# A Gas Chromatography/Electron Ionization–Mass Spectrometry–Selected Ion Monitoring Method for Determining the Fatty Acid Pattern in Food after Formation of Fatty Acid Methyl Esters

SASKIA THURNHOFER AND WALTER VETTER*

Institute of Food Chemistry, University of Hohenheim, Garbenstrasse 28, D-70599 Stuttgart, Germany

A method using gas chromatography/electron ionization−mass spectrometry (GC/EI-MS) in the selected ion monitoring (SIM) mode was developed for the analysis of fatty acids as methyl esters (FAMEs) in order to determine their percentage contribution to the fatty acid profile in food. In the GC/EI-MS-SIM mode, saturated fatty acids were determined with *m*/*z* 87, monoenoic fatty acids were determined with *m*/*z* 74, and polyenoic fatty acids were determined via the sum of *m*/*z* 79 and *m*/*z* 81. The ratios of these fragment ions and the GC retention data provided additional information for tentative structural assignments. The 28 FAME standards tested provided similar results for the novel GC/EI-MS-SIM method and GC/EI-MS in the full scan mode, both of which were slightly worse than GC/flame ionization detection (FID). Analysis of sunflower oil, suet, and cod liver oil verified that both major and minor fatty acids (20−60% and down to 0.001% contribution to the fatty acid pattern) were determined with sufficient quality that justifies application of the GC/EI-MS-SIM method for the analysis of food samples. Furthermore, the method was ∼20- or ∼10-fold more sensitive than GC/EI-MS in the full scan mode or GC/FID, respectively. The method is suited for both quantitative purposes and fatty acid identification in samples where only low amounts of lipids are available.

**KEYWORDS: Fatty acid methyl esters; GC/EI-MS selected ion monitoring mode; food samples**

## INTRODUCTION

Fatty acids (FA) are major components of lipids, which occur in virtually all types of foods. More than 90% of these carboxylic acids are found esterificated as triacylglycerides (*1*). Because of their nutritional relevance, determination of the FA composition is an important task in both routine food analysis and lipid research. For this purpose, the lipids are normally extracted with proper solvents. FAs — the major constituents of most of the lipid components present in food—are then converted into fatty acid methyl esters (FAMEs) and analyzed by gas chromatography/flame ionization detection (GC/FID) (*1*, *2*). The identification of FAMEs by GC/FID analysis is based on identical retention times of standard and a peak in a sample (*3*). Because of the huge structural variety of FAs (chain lengths from four to 28 carbons, 0−6 double bonds including *cis* and *trans* isomers, alkyl branches, and functional groups), coelutions are inevitable on virtually all GC stationary phases. For example, frequently used columns are coated with polar stationary phases consisting of high amounts of cyanopropyl polysiloxane. On these stationary phases, coelutions of the methyl esters of 18:3n-3 and 20:1n-9 have been reported (*2*, *4*). In addition, the branched chain fatty acids (BCFA) a17:0 and i17:0 coelute with 16:1 isomers, which accounts for an important source of errors in the GC/FID determination of both substance classes (*5*−*7*). On the other hand, *cis/trans* isomers and 22:6n-3 and 24:1n-9 are not separated on columns of the carbowax type (*8*, *9*). The low selectivity of GC/FID is particularly disadvantageous for minor FAs. For these reasons, GC/electron ionization−mass spectrometry (EI-MS) analysis in the full scan mode has been used for verification of results obtained by GC/FID. Because of the higher selectivity of this detection method, coeluting pairs of FAs can be identified. However, MS full scan techniques have drawbacks with regard to the proper determination of signal areas of minor components, and they provide relatively poor signal-to-noise (S/N) ratios. To overcome these disadvantages, we developed a GC/EI-MS-selected ion monitoring (SIM) method in order to link the unique selectivity of GC/EI-MS with the high precision and sensitivity of the SIM mode and applied this technique to the analysis of FAs. Only little activity has been observed in this field. Published GC/MS-SIM methods were based on the detection of the molecular ions of FAME (*10*), whereas others suggested *m*/*z* 87 for the determination of 26:0 (*11*). Saturated and one monoenoic acids were determined by using *m*/*z* 74, *m*/*z* 87, and *m*/*z* 55 relative to the abundances of these ions in reference standards (*12*).

The aim of our study was the identification of suitable SIM masses of FAME for the determination of all relevant FAs in

* To whom correspondence should be addressed. Tel: 0049 711 459 4016. Fax: 0049 711 459 4377. E-mail: w-vetter@uni-hohenheim.de.

10.1021/jf051468u CCC: $30.25 
Published on Web 10/12/2005

food. On the basis of these SIM masses, we attempted to determine the FA pattern of different food samples and compared the results with the traditional GC/FID method. It was also our intention to use the SIM technique for the proper determination of minor FAs and for the determination of FAs when only low amounts of sample are available.

## MATERIALS AND METHODS

**Samples and Chemicals.** Sunflower oil (Davert, Senden, Germany), suet (Heess, Stuttgart, Germany), and cod liver oil (Rügen Fisch, Sassnitz, Germany) were used as food samples. A Supelco 37-component FAME mix was from Sigma-Aldrich (Taufkirchen, Germany). Additional standards of FA were from Larodan (Malmö, Sweden).

Cyclohexane (purest; VWR, Darmstadt, Germany) and ethyl acetate (purest, Acros Organics, Geel, Belgium) were combined (1:1, v/v) and distilled to obtain the azeotropic mixture (54:46, v/v). *n*-Hexane [high-performance liquid chromatography (HPLC) gradient grade] and methanol (HPLC gradient grade) were from Fluka (Taufkirchen, Germany). Isooctane (analytical reagent grade) was from Fisher Scientific (Ulm, Germany), isolute-HM-N was from Separtis (Grenzlach-Wyhlen, Germany), and boron trifluoride−methanol complex solution (13−15% $BF_3$ in methanol) was from Riedel-de-Haën (Taufkirchen, Germany).

**Sample Cleanup.** Freeze-dried food samples (~1.0 g) were extracted with an ASE 200 (Dionex, Idstein, Germany) system by using 11 mL extraction cells filled to the brim with approximately 2.0 g of diatomaceous earth (isolute-HM-N). The azeotropic mixture of cyclohexane and ethyl acetate was used as solvent (*13*). The conditions used were as follows: temperature, 80 °C; pressure, 10 MPa; preheat, 0 min; heat, 5 min; static, 10 min; flush, 60%; purge, 120 s; and cycles, 2. Every cell was extracted thrice with a total of 120 mL of solvent. After solvent adjustment to 10 mL, 1 mL was taken for gravimetric determination of the lipid content. The remaining fraction was first condensed with a rotary evaporator (180 mbar, 30 °C) and then evaporated to dryness using a gentle stream of nitrogen.

FAME derivatives were prepared according to official standard procedures (DGF-Einheitsmethode) (*14*, *15*). In brief, 20 mg of fat or oil was treated with 0.5 mL of methanolic KOH (0.5 M) for 5 min/80 °C. After cooling, 1 mL of methanolic $BF_3$ was added and heated for another 5 min/80 °C. Then, the reaction vials were cooled in an ice bath (~10 min), 2 mL of saturated sodium chloride solution and 2 mL of *n*-hexane were added, and the organic phase including the FAMEs was separated and subjected to GC analysis (*14*).

**GC/EI-MS.** A Hewlett-Packard 5890 series II gas chromatograph was used in combination with a 5971A mass selective detector. One microliter of sample dissolved in *n*-hexane was injected with a 7673A autosampler (splitless mode, split opened after 2 min). The injector and transfer line temperatures were kept at 250 and 280 °C. A fused silica capillary column coated with 100% cyanopropyl polysiloxane (CP-Sil 88, 50 m × 0.25 mm i.d., 0.20 $\mu$m $d_f$; Chrompack, Middelburg, The Netherlands) was installed in the GC oven. The carrier gas helium (purity 5.0) was used at a constant flow rate of 1 mL/min. The GC oven program started at 60 °C (hold time 1 min), which was raised at 7 °C/min to 180 °C, at 3 °C/min to 200 °C (hold time 1 min), and finally at 10 °C/min to 230 °C (hold time 10 min). Mass spectra (*m*/*z* 50−450) were recorded at a rate of five scans per second with an ionization energy of 70 eV. The temperature of the ion source was 165 °C. Under these conditions, the (partial) coelutions of FAs in the 37-component FAME mix were observed as follows: 20:3n-3 with 22:1n-9, 20:3n-6 with 22:0, and 18:3n-3 with 20:1n-9.

In GC/EI-MS-SIM mode, the six fragment ions including *m*/*z* 74, *m*/*z* 87, *m*/*z* 81, and *m*/*z* 79 were determined after a solvent delay of 8 min throughout the run. For the determination of BCFA in the presence of monoenoic FAs, we additionally monitored the respective molecular ions *m*/*z* 228 (13:0 isomers) and *m*/*z* 242 (14:0 isomers) from 8 to 16.8 min, *m*/*z* 240 (14:1 isomers) and *m*/*z* 256 (15:0 isomers) from 16.8 to 17.8 min, *m*/*z* 254 (15:1 isomers) and *m*/*z* 270 (16:0 isomers) from 17.8 to 18.9 min, *m*/*z* 268 (16:1 isomers) and *m*/*z* 284 (17:0 isomers) from 18.9 to 19.9 min, *m*/*z* 282 (17:1 isomers) and *m*/*z* 298 (18:0 isomers) from 19.9 to 21.1 min, and *m*/*z* 296 (18:1 isomers) and *m*/*z* 312 (19:0 isomers) from 21.1 to 38.81 min.

Confirmatory measurements were performed with a CP-3800 gas chromatograph connected to a 1200 triplequadrupole MS (Varian, Darmstadt, Germany). The split/splitless injector was operated in splitless mode for 2 min and kept at 250 °C. Separations were achieved on a Factor Four CP-Sil 8MS column (30 m, 0.25 mm i.d., 0.25 $\mu$m $d_f$, Varian) with a constant flow of 1 mL/min helium (purity 5.0) throughout the measurements. The GC oven temperature program started at 60 °C (hold time 1 min), which was then raised at 7 °C/min to 180 °C, then with 3 °C/min to 200 °C (1 min), 10 °C/min to 230 °C (20 min), and 5 °C/min to the final temperature of 300 °C (hold time 7.19 min). The total run time was 70 min. The transfer line was heated to 280 °C. The ion source temperature was set to 200 °C, and the detector voltage was 900 V. After a solvent delay of 5 min, *m*/*z* 50 to *m*/*z* 450 was recorded with an ionization energy of 70 eV.

**GC/FID.** Analyses were carried out with a Hewlett-Packard 5890 series II gas chromatograph equipped with an FID and a split/splitless injector. The injector and detector temperatures were set to 250 and 260 °C, respectively. The injections were performed in splitless mode (split opened after 5 min). Helium 5.0 was used as the carrier gas with a constant column head pressure of 20 psi. Analysis was performed with a 60 m × 0.32 mm i.d. fused silica capillary column coated with 0.2 $\mu$m 95% cyanopropyl/5% methylpolysiloxane (SP-2331, Supelco, Taufkirchen, Germany). The GC oven program started at 100 °C (hold time 2 min), which then was raised at 7 °C/min to 180 °C (hold time 1 min), at 7 °C/min to 220 °C (hold time 4 min), and finally at 5 °C/min to 240 °C (hold time 6 min). The total run time was 39.0 min. The FAMEs were identified by comparison of retention times of standards with peaks in sample. Note that the elution profile was slightly different than on the GC/MS system. Under these conditions, 18:3n-6 eluted before 20:0 and 20:3n-6 eluted before 22:0 whereas 22:1n-9 and 20:3n-3 were not baseline separated when the 37-component FAME mix was injected.

## RESULTS AND DISCUSSION

**Identification of Suitable SIM Masses in the Mass Spectra of FAME.** The mass spectra of FAMEs showed the known strong fragmentation with ion clusters distributed over the entire mass range (**Figure 1a**−**d**). In general, the peak intensities of the fragment ions decreased asymptotically with highest abundance between *m*/*z* 50 and *m*/*z* 100 along with low intensities of the molecular ions (*16*). SIM values in the high mass range could not be used for quantitative purposes since the low relative abundance was connected with significant variations in repetitive analyses. In addition to differences resulting from different chain lengths, the mass spectra also varied in dependence of the number of double bonds. No common ions were present in the higher mass range of FAMEs with the same carbon number but different degree of saturation (**Figure 1a**−**d**) (*17*). The mass spectra of saturated and monoenoic FAMEs were dominated by the representative fragment ion *m*/*z* 74 (**Figures 1a**,**b** and **2a**) (*18*−*20*). However, both classes of FAs showed different fragmentation patterns in the low mass range. While *m*/*z* 87 (for its formation see **Figure 2b**) was found in the mass spectra of both classes, only monoenoic FAs showed a higher proportion of fragment ions such as *m*/*z* 83 and *m*/*z* 97 (**Figure 1a**,**b**). Thus, the contribution of both *m*/*z* 74 and *m*/*z* 87 to the TIC was lower for monoenoic than for saturated FA (**Table 1**).

By contrast, both *m*/*z* 74 and *m*/*z* 87 only played a minor role (0.34−1.5% relative abundance, **Table 1**) in the mass spectra of polyunsaturated fatty acids (PUFA) (**Figure 1c**,**d**). Obviously, methyl esters of PUFAs do not form the radical cation in the headgroup but in the double bond region of the aliphatic chain. The most characteristic fragment ions of PUFA were *m*/*z* 79 and *m*/*z* 81, which most likely arise from cyclohexene and cyclohexadiene radical ions (**Figures 1c**,**d** and

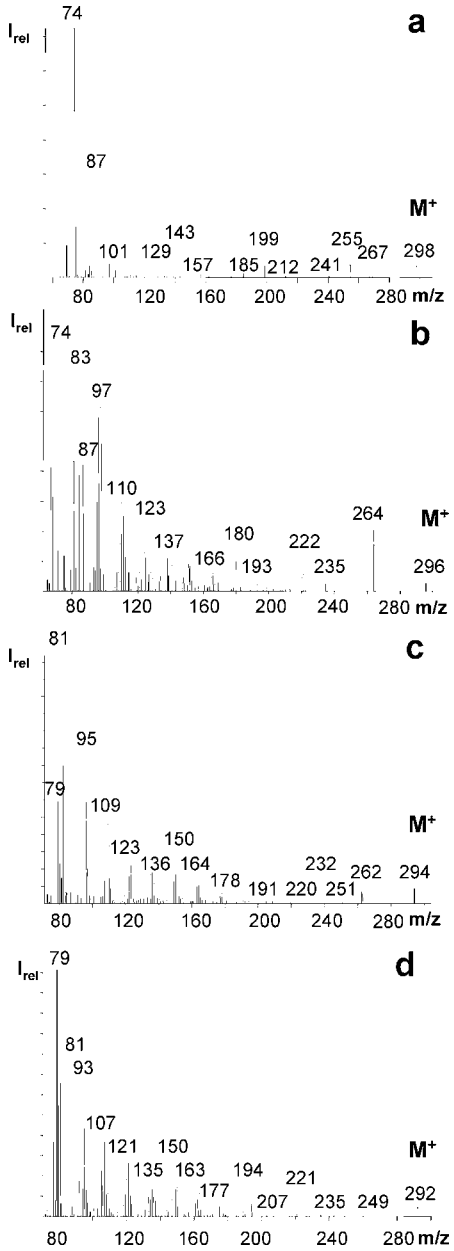


**Figure 1.** GC/EI-MS full scan spectra of the methylesters of (**a**) stearic acid (18:0), (**b**) oleic acid (18:1n-9), (**c**) linoleic acid (18:2n-6), and (**d**) $\gamma$-linolenic acid (18:3n-6).

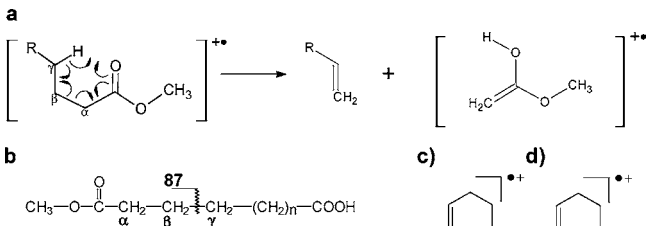


**Figure 2.** Structures of the ions selected for SIM determination of FAs. (**a**) Formation of *m*/*z* 74 via McLafferty rearrangement (note that the hooks represent one-electron movements); (**b**) formation of *m*/*z* 87 by $\gamma$-cleavage; (**c**) proposed structure of *m*/*z* 81 (cyclohexenyl cation); and (**d**) *m*/*z* 79 (cyclohexadienyl cation).

**2c**,**d**) (*21*). Interestingly, the mass spectra of dienoic acids showed a higher abundance of *m*/*z* 81, whereas PUFAs with more double bonds showed a higher abundance of *m*/*z* 79 (**Table 1**).

Our evaluation of the mass spectra shown in **Figure 1** confirmed that even in the low mass range of FAMEs there was no single ion that could be used for SIM determinations of all FAMEs. However, *m*/*z* 74, *m*/*z* 87, *m*/*z* 81, and *m*/*z* 79 together appeared to be suitable and we studied their GC/EI-MS abundances in the full scan mass spectra of a wide range of FAMEs. Although *m*/*z* 74 was almost twice as abundant as *m*/*z* 87 in the mass spectra of staight chain saturated FA (**Table 1**), the latter ion showed lower variance among FAs with different chain lengths (**Figure 3**). Therefore, *m*/*z* 87 was more suitable for the quantification of saturated FAs (**Table 1** and **Figure 3**). Despite some variations in the contribution of individual FA, the mean value of 16.6% covered the range of 15:0 to 23:0 within a margin of ±10% (**Table 1**). Note however that short chain FA and food relevant saturated BCFA showed somewhat lower abundances of both *m*/*z* 87 and *m*/*z* 74, so that these FAs are slightly discriminated when determined with *m*/*z* 87 (**Table 1**).

For the monoenoic FAs, the standard deviation of *m*/*z* 74 and *m*/*z* 87 was very similar (**Figure 3**). Therefore, the percentage contribution of the more abundant ion at *m*/*z* 74 was found more appropriate for GC/EI-MS-SIM determinations. Particularly in the range of food relevant 16:1 and 18:1 isomers, a very constant percentage contribution to the TIC was found (**Table 1**).

As noted above, the ratio of *m*/*z* 79 to *m*/*z* 81 in the mass spectra of PUFA decreased with an increasing number of double bonds (dienoic through hexaenoic FAME); however, the sum of *m*/*z* 81 and *m*/*z* 79 constantly contributed with ~13% to the TIC (**Table 1** and **Figure 3**).

**Factors To Be Included for Determinations and Quality Criteria for Identification.** Because of the relative constant ratios found for the ions in the different types of FAMEs (saturated, monoenoic, and polyenoic), these four ions could be used for determination of the FA composition. Restriction to such a few low mass fragment ions is accompanied with a loss of valuable information as compared to the full scan mode. However, the relative constant ratio between the selected fragment ions allowed for establishing suitable quality criteria in order to enhance the selectivity of the SIM method (**Table 2**). In addition to the retention times (quality criterion 1), saturated FAs were positively identified if criteria were met as follows: (i) the sum of the ion abundances of *m*/*z* 74 and *m*/*z* 87 had to account for more than 90% of the four ions (quality criterion 2), and (ii) the ratio *m*/*z* 87 to *m*/*z* 81 had to be >10 (quality criterion 3). Note that these criteria were also fulfilled for saturated BCFA (see above). Similarly, monoenoic, dienoic, and trienoic acids were positively identified once a respective peak in samples fulfilled the respective criteria (**Table 2**). On the first glance, this procedure may sound complicated but we developed a simple Excel working sheet that allows for automatic testing of any peak detected in a chromatogram for the criteria listed in **Table 2**. By using these criteria, it was possible to assign the group of FA to any of the relevant peaks in standards and food samples (see below).

The respective peak areas had to be multiplied with the following mean factors to obtain the percentage contribution of a FA to the FA pattern of a sample (**Table 2**). For example, in the case of saturated FAs, the peak area of *m*/*z* 87 (16.6% average contribution to TIC) was multiplied with a factor 6.0 to upscale to 100%. Similarly, the factors for monoenoic FA and PUFA were obtained (**Table 2**). Because of some variation of individual FAMEs from the mean factor (see **Tables 1** and

**Table 1.** Structures, Retention Times, and Percentage Contribution of Four *m*/*z* Values to the Total Ion Current (TIC) of the FAMEs[a]

| no. | systematic name | trivial name | empiric formula | $t_R$ (min) | percentage contribution to full scan MS ($n = 6$) (%) | | | | individual factor[b] |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | *m*/*z* 74 | *m*/*z* 87 | *m*/*z* 79 | *m*/*z* 81 | |
| | | | | saturated FAMEs | | | | | |
| 1 | octanoic acid[c] | caprylic acid | 8:0 | 8.92 | 44.04 | **16.46** | 0.13 | 0.19 | **4.95** |
| 2 | decanoic acid[c] | capric acid | 10:0 | 11.72 | 38.08 | **18.44** | 0.15 | 0.51 | **5.26** |
| 3 | undecanoic acid[c] | | 11:0 | 13.03 | 38.82 | **18.89** | 0.15 | 0.47 | **5.03** |
| 4 | dodecanoic acid[c] | lauric acid | 12:0 | 14.40 | 34.61 | **19.21** | 0.19 | 0.48 | **4.90** |
| 5 | tridecanoic acid[c] | | 13:0 | 15.47 | 34.79 | **19.17** | 0.19 | 0.51 | **4.96** |
| 6 | tetradecanoic acid[c] | myristic acid | 14:0 | 16.60 | 32.20 | **19.03** | 0.21 | 0.60 | **5.02** |
| 7 | pentadecanoic acid[c] | | 15:0 | 17.67 | 33.13 | **18.48** | 0.19 | 0.61 | **5.09** |
| 8 | hexadecanoic acid[c] | palmitic acid | 16:0 | 18.70 | 29.11 | **18.08** | 0.22 | 0.66 | **5.22** |
| 9 | heptadecanoic acid[c] | margaric acid | 17:0 | 19.77 | 32.06 | **17.62** | 0.18 | 0.70 | **5.13** |
| 10 | octadecanoic acid[c] | stearic acid | 18:0 | 20.85 | 29.84 | **17.35** | 0.20 | 0.71 | **5.22** |
| 11 | nonadecanoic acid[d] | | 19:0 | 22.00 | 30.38 | **16.89** | 0.19 | 0.75 | **4.85** |
| 12 | eicosanoic acid[c] | arachidic acid | 20:0 | 23.21 | 28.31 | **16.56** | 0.23 | 0.81 | **5.45** |
| 13 | heneicosanoic acid[c] | | 21:0 | 24.74 | 28.17 | **16.04** | 0.20 | 0.86 | **5.50** |
| 14 | docosanoic acid[c] | behenic acid | 22:0 | 25.84 | 25.84 | **15.20** | 0.18 | 0.85 | **5.44** |
| 15 | tricosanoic acid[c] | | 23:0 | 27.42 | 25.93 | **14.81** | 0.18 | 1.11 | **5.85** |
| 16 | tetracosanoic acid[c] | lignoceric acid | 24:0 | 28.56 | 24.63 | **14.73** | 0.25 | 0.96 | **5.93** |
| 17 | pentacosanoic acid[d] | | 25:0 | 29.38 | 24.10 | **14.37** | 0.19 | 0.93 | **6.06** |
| 18 | hexacosanoic acid[d] | cerotic acid | 26:0 | 30.39 | 23.01 | **14.05** | 0.20 | 0.95 | **6.19** |
| 19 | heptacosanoic acid[d] | | 27:0 | 31.39 | 25.23 | **13.50** | 0.21 | 1.13 | **5.78** |
| 20 | octacosanoic acid[d] | montanic acid | 28:0 | 32.59 | 24.34 | **12.88** | 0.18 | 1.03 | **6.06** |
| | | mean and SD | | | 30.33 ± 5.52 | **16.59 ± 1.99** | 0.19 ± 0.03 | 0.74 ± 0.24 | |
| | | | | methyl branched FAMEs | | | | | |
| 21 | 10-methyldodecanoic acid[d] | | a13:0 | 15.12 | 26.08 | **14.50** | 0.25 | 0.92 | **6.28** |
| 22 | 11-methyltridecanoic acid[d] | | a14:0 | 16.24 | 25.66 | **14.37** | 0.26 | 0.93 | **6.26** |
| 23 | 12-methyltetradecanoic acid[d] | | a15:0 | 17.33 | 25.64 | **13.96** | 0.24 | 0.92 | **6.30** |
| 24 | 14-methylhexadecanoic acid[d] | 14-methyl-palmitic acid | a17:0 | 19.41 | 25.26 | **13.26** | 0.23 | 0.95 | **6.64** |
| | | mean and SD | | | 25.66 ± 0.34 | **14.02 ± 0.56** | 0.25 ± 0.01 | 0.93 ± 0.01 | |
| | | | | monoenoic FAMEs | | | | | |
| 25 | 9-tetradecenoic acid[c] | myristoleic acid | 14:1n-5 | 17.54 | **6.81** | 4.29 | 0.90 | 3.07 | **14.65** |
| 26 | 10-pentadecenoic acid[c] | | 15:1n-5 | 18.60 | **6.50** | 4.20 | 0.84 | 3.00 | **15.33** |
| 27 | *trans*-9-hexadecenoic acid[d] | palmitelaidic acid | 16:1n-7t | 19.24 | **5.67** | 3.54 | 0.89 | 2.89 | **19.23** |
| 28 | 9-hexadecenoic acid[c] | palmitoleic acid | 16:1n-7 | 19.39 | **6.08** | 3.74 | 0.88 | 3.10 | **16.24** |
| 29 | 10-pentadecenoic acid[c] | | 17:1n-7 | 20.61 | **5.70** | 3.56 | 0.83 | 2.95 | **17.49** |
| 30 | *trans*-9-octadecenoic[c] | elaidic acid | 18:1n-9t | 21.49 | **5.53** | 3.40 | 0.89 | 3.25 | **18.00** |
| 31 | 9-octadecenoic acid[c] | oleic acid | 18:1n-9c | 21.70 | **5.28** | 3.46 | 0.89 | 3.12 | **18.86** |
| 32 | 11-eicosenoic acid[c,d] | gondoic acid | 20:1n-9 | 24.03 | **4.78** | 3.02 | 0.77 | 2.64 | **21.87** |
| 33 | 13-docosenoic acid[c,d] | erucic acid | 22:1n-9 | 26.57 | **4.68** | 2.77 | 0.70 | 3.19 | **21.05** |
| 34 | 15-tetracosenoic acid[c] | nervonic acid | 24:1n-9 | 28.95 | **4.85** | 2.84 | 0.60 | 2.62 | **21.28** |
| | | mean and SD | | | **5.59 ± 0.72** | 3.48 ± 0.51 | 0.82 ± 0.51 | 2.98 ± 0.21 | |
| | | | | dienoic FAMEs | | | | | |
| 35 | *trans*,*trans*-9,12-octadecadienoic acid[c] | linolelaidic acid | 18:2n-6t | 22.29 | 1.21 | 0.73 | **4.00** | **9.37** | **7.52** |
| 36 | 9,12-octadecadienoic acid[c] | linoleic acid | 18:2n-6c | 22.75 | 1.11 | 0.69 | **3.97** | **9.28** | **7.68** |
| 37 | 11,14-eicosadienoic acid[c] | | 20:2n-6 | 25.31 | 1.27 | 0.67 | **3.54** | **9.49** | **7.91** |
| 38 | 13,16-docosadienoic acid[c] | brassic acid | 22:2n-6 | 27.89 | 1.50 | 0.76 | **3.19** | **9.32** | **8.32** |
| | | mean and SD | | | 1.27 ± 0.17 | 0.71 ± 0.04 | **3.68 ± 0.39** | **9.37 ± 0.09** | |
| | | | | polyunsaturated FAMEs | | | | | |
| 39 | 6,9,12-octadecatrienoic acid[c] | $\gamma$-linolenic acid | 18:3n-6 | 23.59 | 0.89 | 1.76 | **9.07** | **4.86** | **7.55** |
| 40 | 11,14,17-eicosatrienoic acid[c,d] | | 20:3n-3 | 26.69 | 1.21 | 1.20 | **10.19** | **4.75** | **6.69** |
| 41 | 5,8,11,14-eicosatetraenoic acid[c] | arachidonic acid | 20:4n-6 | 26.92 | 1.20 | 0.78 | **9.74** | **3.30** | **7.88** |
| 42 | 5,8,11,14,17-eicosapentaenoic acid[c] | timnodonic acid | 20:5n-3 | 28.21 | 1.11 | 0.64 | **10.69** | **2.46** | **7.69** |
| 43 | 4,7,10,13,16,19-docosahexaenoic acid[c] | | 22:6n-3 | 30.81 | 1.06 | 0.34 | **10.31** | **2.02** | **8.13** |
| | | mean and SD | | | 1.09 ± 0.13 | 0.94 ± 0.55 | **10.00 ± 0.62** | **3.48 ± 1.30** | |

[a] The *m*/*z* values proposed for the determination of the percentage distribution to the FA pattern are in bold type. [b] The individual correction factor is used for quantification. This factor includes a further correction from slight differences between the ratio of the four target ions obtained in the SIM and the full scan mode. [c] Present in the 37-component FAMEs mix. [d] Available as individual standards.

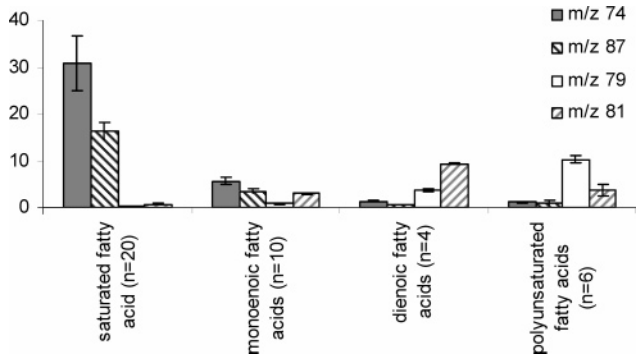


**Figure 3.** Percentage contribution (mean and standard deviation) of *m/z* 74, *m/z* 87, *m/z* 79, and *m/z* 81 to the TIC of saturated, monoenoic, and polyenoic FAs in GC/EI-MS full scan chromatograms.

**2**), we also caluclated individual factors for individual FAs (see **Table 1**, right column).

Different GC detection methods were used for the determination of the abundances of 28 FAMEs present with known concentrations in the 37-component FAME mix (**Table 3**). Only FAMEs that could be determined without interference in all detectors were included (see Materials and Methods for coelutions). The repeatability (typically ~1% deviation) calculated after six injections of the FAME mix was excellent for all methods (**Table 3**). Using GC/FID, all values except for 17:0, 18:1n-9c, and 22:6n-3 deviated ≤5% from the target value. The values determined by our GC/EI-MS-SIM method for individual FAMEs showed a 2-fold worse standard deviation (SD) than the classic GC/FID method (**Table 3**). The results obtained with the individual standards were slightly better than those based on the mean factors (**Table 3**, columns 3 and 4). Given the higher selectivity of GC/MS, the higher deviation as compared to GC/FID was considered to be acceptable for the determination of the FA composition of food. As mentioned above, the type of all FAs was correctly identified on the basis of the quality criteria defined in **Table 2** (data not shown). Because of the relatively uniform response factors for individual FAMEs of the same type, the present GC/EI-MS-SIM method also allowed for the determination of FA for which no standards are available (see next section).

**Determination of the FA Profile in Food Samples.** For further validation of the new GC/EI-MS-SIM method, we analyzed different food samples and compared the results with those obtained by GC/FID and GC/EI-MS in the full scan mode. Comparative analyses of sunflower oil confirmed that both major (contribution up to ~60%) and minor FAs (contribution down to ~0.001%) could be determined with good agreement between GC/FID and GC/EI-MS-SIM except for 18:0, 22:0, and 23:0, which were more relevant when GC/MS was used (**Table 4**). Tentative identification of 18:1n-7 (no standard available) was only possible with GC/MS. Results based on individual factors were closer matching the GC/FID values than GC/EI-SIM based on mean factors. For this reason, we compared our GC/MS data determined with individual factors with GC/FID. For the most FAMEs, the coincidence between GC/FID and GC/EI-MS-SIM was better than compared with GC/EI-MS in the full scan mode.

The suitability of the GC/EI-MS-SIM method was further investigated by the analysis of the more complex FA pattern of suet, which contained relatively high amounts of BCFA. While the contribution of major FAMEs could be determined with all three detection methods (**Table 5**), some BCFA coeluted with monoenoic FAs and a15:0 and a17:0 could not be determined individually with GC/FID but with GC/MS (**Table 5**). Partial coelution was also observed when GC/MS was used. However, the presence of *m/z* 74 and *m/z* 87 in both types of FAs hindered a proper determination of the coeluting FAMEs. For selective determination of BCFA, we additionally monitored the molecular ions of i15:0 and a15:0 (*m/z* 256) and 14:1 isomers (*m/z* 240) and, in a later time window, a/i17:0 (*m/z* 284) and 16:1 isomers (*m/z* 268) (see Materials and Methods). **Figure 4** illustrates the partial coelution of 16:1n-7 and a17:0. By means of GC/FID, it was not possible to identify either 16:1n-7 or a17:0, which could be done with the help of GC/MS. Note also the minor isomer (marked with an asterisk in **Figure 4**) of 16:1n-7, which eluted slightly after i17:0 from the CP-Sil 88 column. From the relative abundance of the molecular ions, the contribution of the coeluting FAME to peak in the chromatogram could be established (**Figure 4** and **Table 5**).

Final testing of the method was performed with cod liver oil due to both high proportions of FAME not found in FAME standard mixes (*9*). Twenty-seven FAMEs could be studied and compared (**Table 6**). For most FAs, the agreement between GC/EI-MS-SIM and GC/FID was acceptable. However, the contribution of DHA (22:6n-3) to the FA pattern was either underestimated by GC/EI-MS-SIM or DHA was interfered when GC/FID was used (**Table 3**).

The striking parameter that pointed toward the SIM method was the improved sensitivity as compared to GC/EI-MS in the full scan mode. This parameter was determined by the accurate determination of a standard consisting of four anteiso-FAs. In the SIM mode, the limit of quantification (LOQ was defined as S/N = 10) was 20 pg for the FA with the lowest response whereas the LOQs of GC/FID and GC/EI-MS in the full scan mode were ~10- and ~20-fold worse. The low LOQ of the GC/EI-MS-SIM method along with the high selectivity enabled the determination of several low minor FAs (12:0, i13:0, a13:0, i14:0, and a15:0, **Table 6**), which could not be determined with GC/FID and GC/MS in the full scan mode. This illustrates the advantage of the GC/EI-MS-SIM mode for the correct determination of minor FAME in food samples as well as for the detection and quantification of FAs from contamination such as by food-borne bacterial pathogens (*22*).

**Verification of the Method.** Our new SIM method allowed for the proper determination of both major and minor FAs in different food samples. To verify the suitability of the method, we used a second GC/EI-MS system (Varian 1200). Because of different GC/EI-MS parameters (e.g., ion source design and temperature, GC column, etc.) the factors for the three types of FAs were different on both instruments. Nevertheless, the relative contribtuion of the suggested ions to the TIC was also relatively constant for FAs of the same type (saturated, monoenoic, and PUFA) but with different chain lengths. On this instrument, we determined the following mean factors: 7.8 for saturated FAME, 21.2 for monoenoic acids, 10.0 for dienoic acids, and 9.3 for trienoic acids. In each case, the factors on the Varian 1200 instrument were ~1.3-fold higher than on the 5971 MSD, which means that the proposed ions were by the same factor lower abundant in the GC/EI-MS with the second instrument. Thus, the factors should be individually established on other instruments or when other experimental methods are applied. This should also be taken into account when the ion source gets significantly contaminated.

GC/EI-MS in the SIM mode was shown to be a suitable tool for the determination of the percentage contribution of known and unknown FAMEs to the FA pattern of food. GC/EI-MS-SIM provided similar results, but the values did not fully match those obtained with GC/FID in the analysis of standard FAs in

**Table 2.** Quality Criteria Established for the Classification of Unknown FAME without Mass Spectra in a GC/EI-MS-SIM Chromatogram

| no. of double bonds | quality criterion 1 | quality criterion 2 | quality criterion 3 | *m/z* values for quantification | mean multiplication factor |
|---|---|---|---|---|---|
| 0 | $t_R$ | $[m/z\ (74+87)]/\text{TIC} \geq 0.9$ | $m/z\ 87/81 > 10$ | $m/z\ 87$ | 6.0 |
| 1 | $t_R$ | $[m/z\ (74+87)]/\text{TIC} \approx 0.75$ | $m/z\ 87/81 = 0.8$–10 | $m/z\ 74$ | 17.9 |
| 2 | $t_R$ | $m/z\ (79)/\text{TIC} \leq 0.3$<br>$m/z\ (81)/\text{TIC} \geq 0.6$ | $m/z\ 87/81 < 0.8$ | $m/z\ (79+81)$ | 7.7[a] |
| 3 | $t_R$ | $m/z\ (79)/\text{TIC} \geq 0.45$<br>$m/z\ (81)/\text{TIC} \leq 0.3$ | $m/z\ 87/81 < 0.8$ | $m/z\ (79+81)$ | 7.4[a] |

[a] A factor of 7.6 was used for the determination of di- to hexaenoic FAs in food.

**Table 3.** Quantitative Determination of FAs in a Standard Mix[a] with GC/EI-MS in Full Scan and SIM Modes as Well as GC/FID

| | scan ($n=6$) (%) | | SIM ($n=6$) (%) based on mean factor | | SIM ($n=6$) (%) based on individual factors | | FID ($n=6$) (%) | |
|---|---|---|---|---|---|---|---|---|
| FAME | AV | SD | AV | SD | AV | SD | AV | SD |
| 10:0 | 0.92 | 0.01 | 0.91 | 0.00 | 0.86 | 0.01 | 0.98 | 0.07 |
| 11:0 | 1.03 | 0.02 | 1.11 | 0.01 | 1.01 | 0.02 | 0.99 | 0.06 |
| 12:0 | 0.92 | 0.01 | 0.97 | 0.00 | 0.86 | 0.01 | 1.00 | 0.04 |
| 13:0 | 1.07 | 0.02 | 1.20 | 0.01 | 1.07 | 0.01 | 1.03 | 0.03 |
| 14:0 | 1.00 | 0.02 | 1.05 | 0.00 | 0.96 | 0.01 | 1.04 | 0.02 |
| 14:1n-5 | 1.04 | 0.01 | 1.22 | 0.01 | 1.07 | 0.01 | 0.98 | 0.02 |
| 15:0 | 1.09 | 0.02 | 1.23 | 0.00 | 1.13 | 0.02 | 1.03 | 0.01 |
| 15:1n-5 | 1.10 | 0.02 | 1.21 | 0.01 | 1.11 | 0.02 | 1.03 | 0.01 |
| 16:0 | 0.94 | 0.01 | 0.97 | 0.01 | 0.91 | 0.01 | 1.04 | 0.01 |
| 16:1n-7 | 1.06 | 0.01 | 1.14 | 0.01 | 1.11 | 0.03 | 0.97 | 0.01 |
| 17:0 | 0.84 | 0.02 | 0.95 | 0.00 | 0.88 | 0.02 | 0.77 | 0.01 |
| 17:1n-7 | 1.14 | 0.02 | 1.13 | 0.01 | 1.19 | 0.02 | 1.04 | 0.01 |
| 18:0 | 1.06 | 0.01 | 1.14 | 0.00 | 1.08 | 0.02 | 1.03 | 0.01 |
| 18:1n-9tr | 1.16 | 0.01 | 1.15 | 0.01 | 1.24 | 0.02 | 1.02 | 0.01 |
| 18:1n-9c | 1.06 | 0.02 | 0.96 | 0.00 | 1.08 | 0.02 | 1.06 | 0.01 |
| 18:2n-6tr | 1.09 | 0.02 | 0.96 | 0.00 | 1.02 | 0.02 | 1.03 | 0.01 |
| 18:2n-6c | 1.09 | 0.03 | 0.95 | 0.01 | 1.02 | 0.03 | 1.03 | 0.01 |
| 20:0 | 1.02 | 0.01 | 1.06 | 0.00 | 1.05 | 0.03 | 1.02 | 0.02 |
| 18:3n-6 | 0.99 | 0.01 | 1.00 | 0.01 | 1.06 | 0.04 | 0.95 | 0.01 |
| 21:0 | 1.05 | 0.01 | 1.11 | 0.00 | 1.11 | 0.04 | 1.03 | 0.02 |
| 20:2n-6 | 1.07 | 0.01 | 0.92 | 0.01 | 1.02 | 0.03 | 1.01 | 0.02 |
| 20:4n-6 | 0.95 | 0.01 | 0.85 | 0.00 | 0.94 | 0.03 | 1.02 | 0.02 |
| 23:0 | 0.95 | 0.05 | 0.95 | 0.01 | 1.01 | 0.04 | 0.97 | 0.03 |
| 22:2n-6 | 1.02 | 0.03 | 0.83 | 0.00 | 0.96 | 0.03 | 0.97 | 0.02 |
| 20:5n-3 | 1.01 | 0.01 | 0.90 | 0.01 | 0.97 | 0.02 | 1.03 | 0.02 |
| 24:0 | 0.87 | 0.06 | 0.83 | 0.01 | 0.89 | 0.04 | 0.98 | 0.04 |
| 24:1n-9 | 0.91 | 0.06 | 0.79 | 0.00 | 1.00 | 0.03 | 0.99 | 0.04 |
| 22:6n-3 | 0.83 | 0.02 | 0.69 | 0.01 | 0.78 | 0.01 | 0.80 | 0.02 |
| AV ± SD | 1.010 | 0.087 | 1.005 | 0.143 | 1.014 | 0.105 | 1.001 | 0.065 |

[a] The standard included 37 compounds with given percentual contribution; 4:0, 6:0, and 8:0 were not included in this study; 18:3n-3, 20:1n-9, 20:3n-3, 20:3n-6, 22:0, and 22:1n-9 could not be studied due to coelutions (see Materials and Methods). Relative abundances of individual FA (2, 4, or 6%) were normalized to 1% by dividing the peak area with the respective percentage. After this procedure, all peaks should have the same intensity (=1.0).

simple fats. Note that all measurements are relative measurements (100% method), which means that differences in the contribution of one FA to the FA profile will affect the contribution of all other FAs. Therefore, a more detailed evaluation cannot be carried out unless quantitative determinations are carried out, which require the use of internal standards.

Nevertheless, the new GC/EI-MS-SIM method was superior to GC/FID for the determination of minor FAs such as BCFA. Furthermore, the sensitivity was 1 order of magnitude better as compared to GC/EI-MS in the full scan mode and GC/FID. In combination with the quality criteria developed as well, the unique selectivity of GC/EI-MS could be reached although only low mass fragment ions were selected for the determinations. On the basis of these quality criteria, the identity of unknown

**Table 4.** Determined Percentage Contribution of Sunflower Oil with GC/FID and GC/EI-MS in Full Scan and SIM Mode

| FAME | scan (%) | SIM (%) based on mean factor | SIM (%) based on individual factors | FID (%) |
|---|---|---|---|---|
| 12:0 | ND[a] | 0.002 | 0.001 | ND[a] |
| 13:0 | ND[a] | 0.004 | 0.003 | ND[a] |
| 14:0 | 0.06 | 0.08 | 0.07 | 0.08 |
| 15:0 | 0.01 | 0.02 | 0.01 | 0.01 |
| 16:0 | 6.32 | 7.02 | 6.76 | 6.14 |
| 16:1n-9 | 0.02 | 0.04 | 0.03 | 0.02 |
| 16:1n-7 | 0.09 | 0.12 | 0.10 | 0.09 |
| 17:0 | 0.05 | 0.07 | 0.06 | 0.04 |
| 17:1n-7 | 0.03 | 0.04 | 0.03 | 0.03 |
| 18:0 | 4.62 | 5.46 | 4.69 | 4.10 |
| 18:1n-9 | 32.41 | 31.11 | 32.38 | 30.28 |
| 18:1n-7[b] | 0.62 | 0.80 | 0.83 | 0.54 |
| 18:2n-6 | 53.77 | 53.06 | 53.02 | 56.76 |
| 20:0 | 0.42 | 0.22 | 0.20 | 0.28 |
| 22:0 | 1.17 | 1.49 | 1.33 | 0.72 |
| 23:0 | 0.05 | 0.07 | 0.07 | 0.03 |
| 24:0 | 0.35 | 0.43 | 0.42 | 0.30 |

[a] Not detected. [b] No standard available, peak assignment due to GC/MS isomer identification.

**Table 5.** Determined Percentage Contribution of Suet with GC/FID and GC/EI-MS in Full Scan and SIM Modes

| FAME | scan (%) | SIM (%) based on mean factor | SIM (%) based on individual factors | FID (%) |
|---|---|---|---|---|
| 10:0 | 0.03 | 0.04 | 0.04 | 0.06 |
| 12:0 | 0.05 | 0.07 | 0.06 | 0.08 |
| i14:0 | 0.05 | 0.07 | 0.07 | 0.07 |
| 14:0 | 2.55 | 2.96 | 2.62 | 2.81 |
| i15:0 | 0.19 | 0.26 | 0.29 | 0.23 |
| a15:0 | 0.20 | 0.23 | 0.25 | 0.65 |
| 14:1n-5 | 0.33 | 0.45 | 0.39 | |
| 15:0 | 0.45 | 0.61 | 0.54 | 0.47 |
| i16:0 | 0.28 | 0.37 | 0.42 | 0.30 |
| 16:0 | 24.26 | 24.42 | 22.50 | 25.80 |
| i17:0 | 0.37 | 0.45 | 0.52 | 0.36 |
| 16:1n-7 | 3.12[a] | 2.78 | 2.67 | 3.32[a] |
| a17:0 | | 0.81 | 0.95 | |
| 17:0 | 1.33 | 1.57 | 1.42 | 1.26 |
| 18:0 | 24.21 | 25.42 | 23.39 | 23.15 |
| 18:1n-9 | 38.67 | 36.21 | 40.37 | 37.59 |
| 18:2n-6 | 3.63 | 2.93 | 3.14 | 2.86 |
| 20:0 | 0.28 | 0.36 | 0.35 | 0.24 |

[a] Coelution.

FAMEs in a sample can be established. For this reason, the method is also suggested for the analysis of samples with very low FA content. Instead of the lower sensitive full scan method, the SIM method will provide excellent results for low concentrated FAMEs. By simple recording of *m/z* 74, *m/z* 87, *m/z* 79, and *m/z* 81, all FAs can be detected in food and biological samples. Determination of the relative abundances of the suggested SIM masses can be used to determine the degree of

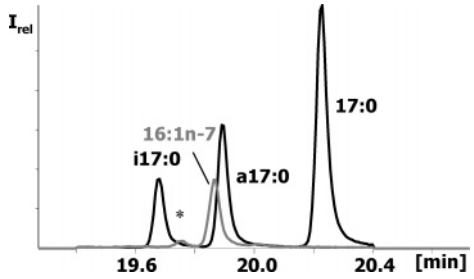


**Figure 4.** GC/MS chromatogram (part) of *m*/*z* 284 (17:0-ME isomers) and *m*/*z* 268 (16:1-ME isomers) in suet.

**Table 6.** Percentage Contribution of Individual FAs to the FA Pattern of Cod Liver Oil as Determined with GC/FID, as Well as GC/EI-MS in Full Scan and SIM Modes

| FAME | scan (%) | SIM (%) based on mean value | SIM (%) based on individual factors | FID (%) |
|---|---|---|---|---|
| 12:0 | ND[a] | 0.05 | 0.04 | ND[a] |
| i13:0 | ND[a] | 0.01 | 0.01 | ND[a] |
| a13:0 | ND[a] | 0.005 | 0.005 | ND[a] |
| 13:0 | ND[a] | 0.02 | 0.02 | ND[a] |
| i14:0 | ND[a] | 0.04 | 0.04 | ND[a] |
| 14:0 | 3.28 | 4.72 | 3.82 | 2.87 |
| i15:0 | 0.36 | 0.51 | 0.52 | 0.38[b] |
| a15:0 | 0.05 | 0.08 | 0.08 | ND[a] |
| 15:0 | 0.46 | 0.71 | 0.58 | 0.50 |
| i16:0 | 0.21 | 0.23 | 0.24 | 0.20[b] |
| 16:0 | 14.17 | 16.67 | 14.03 | 12.60 |
| 16:1n-9 | 0.64 | 1.15 | 0.80 | 0.80 |
| 16:1n-7 | 6.17 | 8.81 | 7.73 | 5.20 |
| 17:0 | 0.27 | 0.57 | 0.47 | 0.9 |
| i18:0 | ND[a] | 0.03 | 0.03 | ND[a] |
| 17:1 | 0.64 | 0.88 | 0.83 | 0.80 |
| 18:0 | 3.20 | 4.12 | 3.46 | 2.90 |
| 18:1n-9 | 24.15 | 24.21 | 24.67 | 24.45 |
| 18:1n-7[c] | 6.40 | 7.28 | 7.42 | 5.0 |
| 18:2n-6 | 3.87 | 3.55 | 3.43 | 3.3 |
| 18:3n-3 | 3.54 | 3.35 | 3.24 | 2.6 |
| 20:1n-9 | | 0.54 | 0.64 | 0.9 |
| 18:4n-3 | 2.09 | 2.59 | 2.51 | 2.2 |
| 20:2n-2 | 0.66 | 0.64 | 0.64 | 0.70 |
| 20:4n-3 | 0.74 | 0.86 | 0.86 | 0.80 |
| 20:5n-3 | 10.20 | 9.80 | 10.44 | 10.7 |
| 22:6n-3 | 14.24 | 12.34 | 12.75 | 16.7 |

[a] Not detected. [b] Determined after verification by using GC/MS. [c] No standard available, peak assignment due to GC/MS isomer identification.

saturation. Consequently, the method is recommended for both the determination of the relative abundance of FAME in food and the peak identification. In this way, more information can be obtained from the low mass fragments of FAMEs in comparison to the remote site derivatization mode (DMOX derivatives and picolinyl esters of FAs (*1*, *19*), which yield the same low-mass fragment ions for all FAs irrespective of the number of double bonds. For instance, picolinyl esters are dominated by *m*/*z* 92 and *m*/*z* 108 whereas DMOX derivatives generally contain *m*/*z* 113 and *m*/*z* 126 (data not shown). This prevents the identification of coeluting FAs, which at least in part can be identified with our GC/EI-MS SIM method using FAME. Additional SIM values of the molecular ions of particularly interesting FA can be used to determine coeluting FAME as was demonstrated for BCFA.

## ACKNOWLEDGMENT

We thank Roland von der Recke from our institute for the recording of mass spectra on the Varian 1200 instrument.

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**Received for review June 22, 2005. Revised manuscript received August 29, 2005. Accepted September 4, 2005. We are grateful for financial support from the German Scientific Foundation (DFG).**

JF051468U