# Determination of Fatty Acid Methyl Esters by GCxGC-TOFMS

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## 1. Introduction

Composition of fatty acids is an important factor influencing the nutritional value of food products. Animal fats contain a high proportion of saturated fatty acids that contribute to the increase of the level of LDL cholesterol (Low Density Lipoproteins bonded cholesterol) in blood, which leads to the risk of atherosclerosis. On the other hand, unsaturated fatty acids, especially polyunsaturated isomers, decrease the levels of LDL cholesterol and therefore have a positive effect on the cardiovascular system.

Naturally present unsaturated fatty acids possess almost exclusively cis double bonds. Trans Fatty Acids (TFAs), being a product of enzymatic hydrolysis in the cow stomach, can occur in negligible amounts in milk fat. However, a more significant source in diet comes from margarines, where TFAs are formed by industrial hydrogenation of vegetable oils. Unlike cis fatty acids, trans fatty acids have negative health effects. Similarly to saturated isomers, the consumption of TFAs increases the risk of atherosclerosis. Toxicological studies have suggested an increased risk of diabetes type II related to TFA intake. Other effects such as cancer of the colon and negative impact on prenatal and newborn health are also suspected.

Depending on the production technology, contents of TFAs can be very high in margarines (up to 30% of total FAs), and also in biscuits and pastry products (up to 50%) made from poor quality raw materials. It is therefore an interest of national food monitoring programs to map the sources of TFAs and subsequently regulate the maximum content of TFAs in food products.

A common method of analysis of fatty acids after their derivatization to methyl esters is gas chromatography with a flame ionization detector (GC-FID). In food samples the number of fatty acids isomers differing by the number and position of double bonds can be high, which leads to complexity of chromatograms and possible coelutions.

In this note, comprehensive two-dimensional gas chromatography (GCxGC) has been applied for the analysis of fatty acids methyl esters. In GCxGC, two columns of different selectivity are connected via a modulator. The modulator repeatedly focuses a small portion of the first column eluate and injects it onto the second column. The second column, being very short and narrow, performs a flash (several seconds) separation for each modulation portion injected. Overall separation capacity of the system is greatly enhanced compared to one-dimensional separation.

## 2. Experimental Conditions

*Samples*

- Standard mixture of 37 fatty acid methyl esters (FAMEs) in hexane. 60 to 180 µg/mL each
- Extract of flaky pastry, derivatized, in hexane
- Extract of egg, derivatized, in hexane

*Analysis Conditions (GC-TOFMS)*

| | |
|---|---|
| GC Parameters: | Agilent 6890N Gas Chromatograph |
| Injection: | 1 µL split 1:50, 250°C |
| Column: | DB-5MS 30 m x 0.25 mm x 0.25 µm (Agilent, USA) |
| Carrier Gas: | Helium, 1 ml/min, constant flow |
| Oven Program: | 40°C, 2 min, 30°C/min to 160°C, 2°C/min to 300°C, hold 5 min |
| Transfer Line Temp: | 240°C |
| Total Run Time: | 83.5 minutes |
| | |
| MS Parameters: | LECO Pegasus® 4D GCxGC-TOFMS |
| Ionization: | Electron Ionization at 70 eV |
| Source Temp: | 220°C |
| Stored Mass Range: | 45 to 650 u |
| Acquisition Rate: | 2 spectra/second |

*Analysis Conditions (GCxGC-TOFMS)*

| | |
|---|---|
| GC Parameters: | Agilent 6890N Gas Chromatograph equipped with a LECO GCxGC Thermal Modulator and Secondary Oven |
| Injection: | 1 µL split 1:50, 250°C |
| Primary Column: | DB-5MS 30 m x 0.25 mm x 0.25 µm (Agilent, USA) |
| Secondary Column: | BPX-50 2 m x 0.1 mm x 0.1 µm (SGE, Australia) |
| Carrier Gas: | Helium, 1 ml/min, constant flow |
| Primary Oven Program: | 40°C, 2 min, 30°C/min to 160°C, 2°C/min to 300°C, hold 5 min |
| Secondary Oven Program: | 45°C, 2 min, 30°C/min to 165°C, 2°C/min to 305°C, hold 5 min |
| Modulator Temperature Offset (above primary oven): | 30°C |
| Modulation Time: | 5 seconds |
| Hot Pulse Time: | 1 second |
| Transfer Line Temp: | 240°C |
| Total Run Time: | 83.5 minutes |
| | |
| MS Parameters: | LECO Pegasus 4D GCxGC-TOFMS |
| Ionization: | Electron Ionization at 70 eV |
| Source Temp: | 220°C |
| Stored Mass Range: | 45 to 650 u |
| Acquisition Rate: | 100 spectra/second |

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## 3. Results and Discussion

### Method Optimization

Two different sets of columns were tested. In the first approach a 2 m (0.1 mm x 0.1 µm) Supelco wax column was used for the second dimension. This column is highly polar, therefore there was a good presumption that FAMEs would be separated based on the degree of saturation. A good second dimension separation was indeed achieved under these conditions, as seen in Figure 1A. In addition, a structured contour plot, where FAMEs were ordered based on the carbon number (X-axis, volatility) and number of double bonds (Y-axis, polarity), was obtained. However, when using a wax column for the second dimension the final plateau of the temperature program had to be limited to 250°C, which is the maximum operating temperature of this column. This resulted in the C21-C24 FAMEs eluting under isothermal conditions. Therefore, the elution pattern in the contour plot is not linear in this area.

Using a BPX-50 (2 m x 0.1 mm x 0.1 µm), which has a higher maximum operating temperature, all the FAMEs were eluted within the ramp of the temperature program and a linear elution pattern was achieved starting with C15 (Figure 1B). The nonlinear elution behavior of the FAMEs prior to about C15 is from the faster oven ramp used at the beginning of the run. Although a slower temperature program could be used in that region, the run time would be increased significantly.

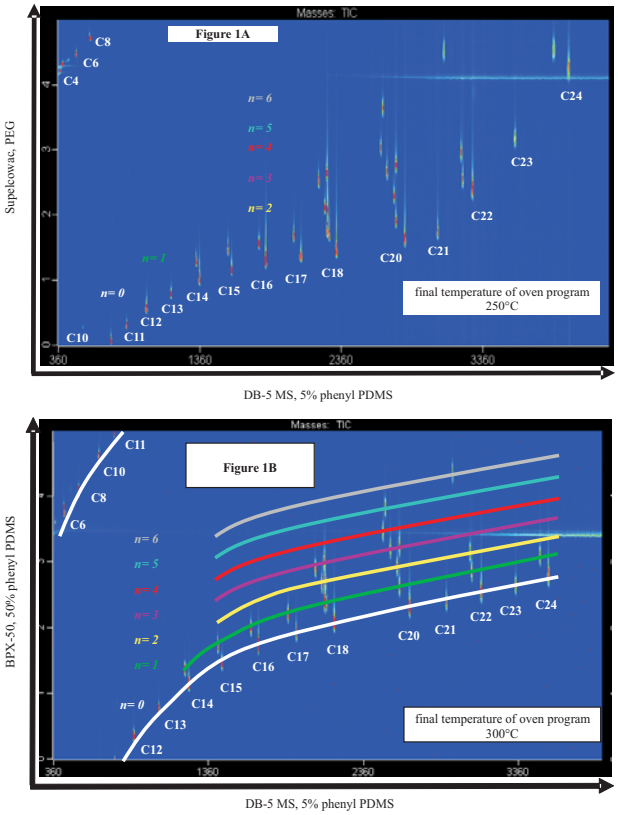


***Figure 1: Contour plot of mixed standard of FAMEs.***
***A - Second dimension Supelcowax (2 m x 0.1 mm x 0.1 µm), temperature program up to 250°C***
***B - FINAL CONDITIONS: Second dimension BPX-50 (2 m x 0.1 mm x 0.1 µm), temperature program up to 300°C***

### *Comparison of 1D and GCxGC Separations*

To compare the separations by one-dimensional chromatography and GCxGC, we focused on the elution area of C18 FAMEs, where FAMEs differing by number as well as position of double bonds were present. In Figure 2, the elution area of C18 FAMEs is shown for one-dimensional GC-TOFMS analysis. As can be seen from this figure, gamma-Linolenic (18:3 ω6, cis), Oleic (18:2 ω9, cis) and Linoleaidic acid (18:1 ω6, trans) methyl esters were not separated. However, despite the close coelution and similar mass spectra, the deconvolution algorithm of ChromaTOF software was still able to separate mass spectra and identify those three peaks correctly (Figure 4; next page).

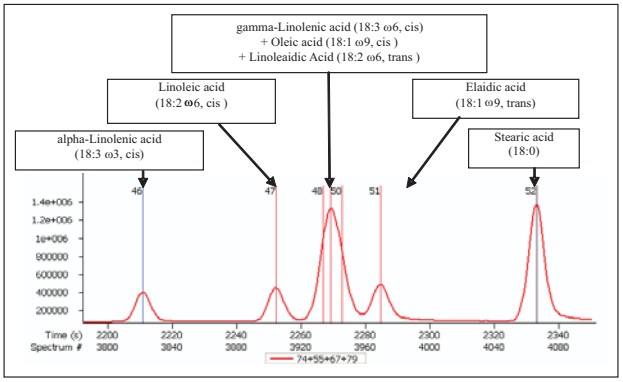


***Figure 2: 1D GC-TOFMS analysis of standard. FAMEs separation in C18 elution range.***

When GCxGC separation was applied (with the same primary oven temperature program and analysis time), all the C18 FAMEs are well separated with the elution pattern mentioned above, i.e. the higher the number of double bonds, the higher in the contour plot the compound is eluted (Figure 3).

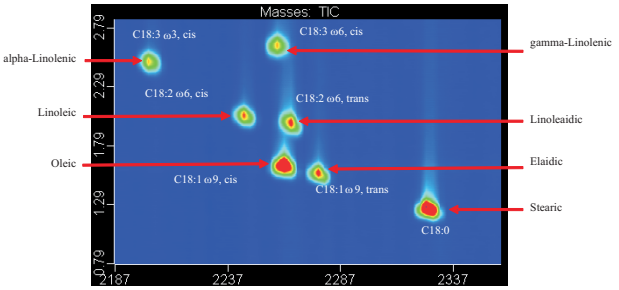


***Figure 3: GCxGC-TOFMS analysis of standard. FAMEs separation in C18 elution range.***

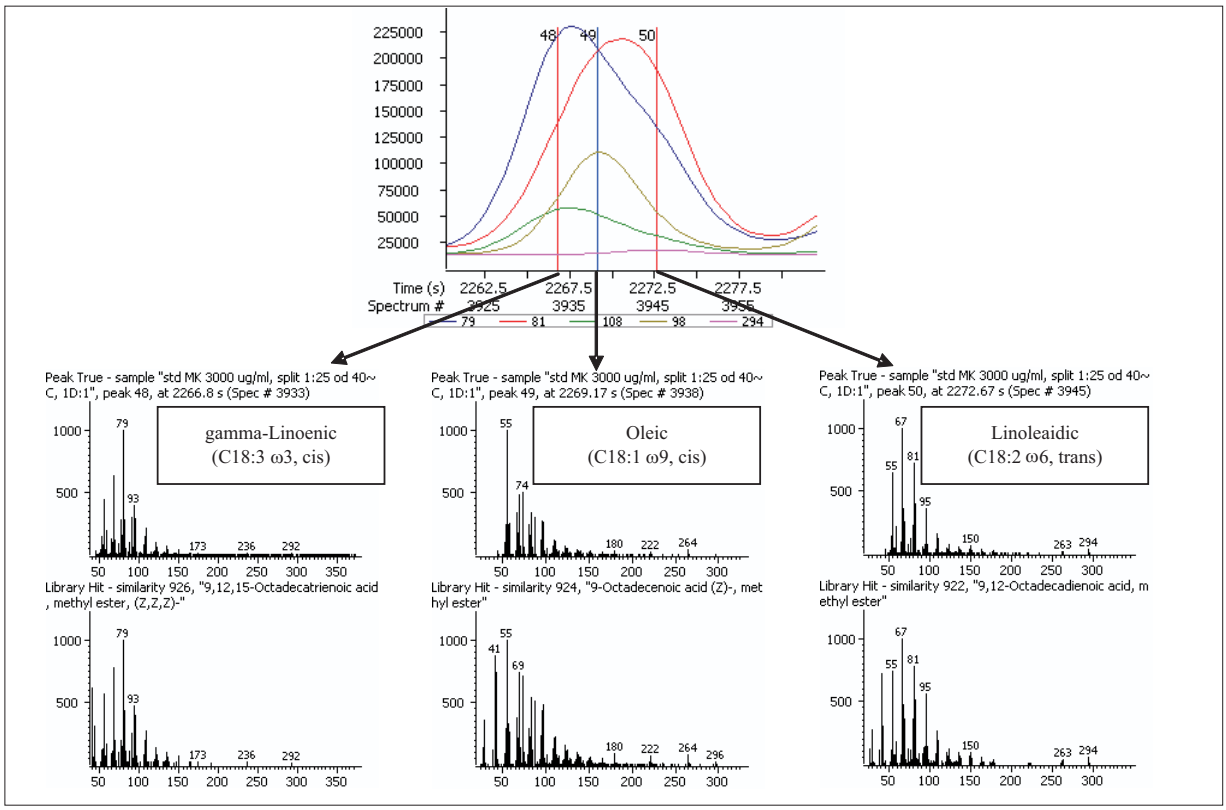


***Figure 4: 1D GC-TOFMS analysis. Mass spectral deconvolution of gamma-Linolenic, Oleic and Linoleaidic acid methyl esters.***

*Analysis of Real Samples*

In Figure 5, the C18 FAMEs sections of the contour plots from the flaky pastry and egg samples analyses are displayed. One of the advantages of GCxGC is that the contour plot quickly gives information about the compounds present in the samples. In this particular case, it is obvious that Linoleic (18:2 ω6, cis), Oleic (18:1 ω9, cis), Elaidic (18:1 ω9, trans) and Stearic (18:0) acids are present in the flaky pastry sample. From qualitative point of view, the egg sample contains the same C18 fatty acids. However, the amount of trans Elaidic acid is obviously lower.

All 37 fatty acids were quantified after using the automated calibration function of the ChromaTOF software. Results are given in Table 1; see page 4.

## 4. Conclusions

A GCxGC-TOFMS method for the analysis of fatty acid methyl esters, including trans fatty acids, has been developed. In comparison with one-dimensional chromatography, GCxGC brought significantly improved separation. All 37 FAMEs were baseline separated by the developed method. In addition, clear structure was obtained in the contour plot, where FAMEs were distributed based on carbon number on the X-axis (volatility) and based on number of double bonds on the Y axis (polarity). This utilization of two-dimensional separation space gives the potential to separate even more complex samples, containing many different FAME isomers.

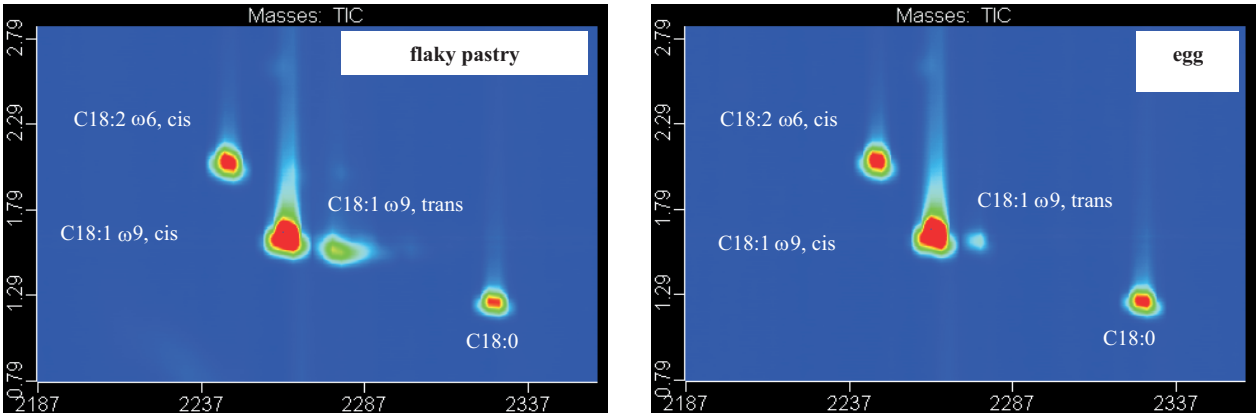


***Figure 5: GCxGC-TOFMS analysis of flaky pastry and eggs. C18 FAMEs range of contour plot.***

Delivering the Right Results

**Table I: Quantification of fatty acids in flaky pastry and egg samples.**

| Expected Analyte R.T. (s) | Name | Name (in symbols) | Quant Masses | Concentration (µg/ml) | |
|---|---|---|---|---|---|
| | | | | Flaky pastry | Egg |
| 430 , 3.800 | Hexanoic acid, methyl ester (Caproic) | 6:0 | 87 | 0.14 | 0.02 |
| 525 , 4.120 | Octanoic acid, methyl ester (Caprylic) | 8:0 | 87 | 0.84 | 0.08 |
| 655 , 4.630 | Decanoic acid, methyl ester (Capric) | 10:0 | 87 | 0.78 | 0.10 |
| 750 , 4.970 | Undecanoic acid, methyl ester | 11:0 | 74 | 0.06 | Not Found |
| 880 , 0.380 | Dodecanoic acid, methyl ester (Lauric) | 12:0 | 74 | 5.70 | 0.05 |
| 1040 , 0.780 | Tridecanoic acid, methyl ester | 13:0 | 74 | 0.06 | Not Found |
| 1210 , 1.370 | cis-9-Tetradecenoic acid, methyl ester (Myristoleic) | 14:1 ω5, cis | | Not Found | 0.65 |
| 1230 , 1.180 | Tetradecanoic acid, methyl ester (Myristic) | 14:0 | 74 | 6.96 | 3.68 |
| 1420 , 1.750 | cis-10-Pentadecenoic acid, methyl ester | 15:1 ω5, cis | | Not Found | Not Found |
| 1450 , 1.460 | Pentadecanoic acid, methyl ester | 15:0 | 74 | 0.27 | 0.48 |
| 1630 , 2.020 | cis-9-Hexadecenoic acid, methyl ester (Palmitoleic) | 16:1 ω7, cis | 55 | 0.80 | 27.31 |
| 1680 , 1.760 | Hexadecanoic acid, methyl ester (Palmitic) | 16:0 | 74 | 173.24 | 242.44 |
| 1875 , 2.220 | cis-10-Heptadecenoic acid, methyl ester | 17:1 ω7, cis | | Not Found | Not Found |
| 1925 , 1.940 | Heptadecanoic acid, methyl ester | 17:0 | 74 | 0.42 | 1.63 |
| 2050 , 2.900 | all cis-9,12,15-Octadecatrienoic acid, methyl ester (alfa-Linolenic) | 18:3 ω3, cis | | Not Found | 1.05 |
| 2090 , 2.680 | cis, cis-9,12-Octadecadienoic acid, methyl ester (Linoleic) | 18:2 ω6, cis | 67 | 58.79 | 22.00 |
| 2105 , 2.430 | cis-9-Octadecenoic Acid (Oleic) | 18:1 ω9, cis | 55 | 153.47 | 143.37 |
| 2105 , 3.020 | all cis-6,9,12-Octadecatrienoic acid, methyl ester (gamma-Linolenic) | 18:3 ω6, cis | 79 | 2.25 | 6.02 |
| 2110 , 2.740 | trans, trans-9,12-Octadecadienoic acid, methyl ester (Linoleaidic) | 18:2 ω6, trans | 67 | 3.80 | 0.26 |
| 2125 , 2.350 | trans-9-Octadecenoic acid, methyl ester (Elaidic) | 18:1 ω9, trans | 55 | 31.08 | 22.98 |
| 2170 , 2.110 | Octadecanoic acid, methyl ester (Stearic) | 18:0 | 74 | 35.13 | 121.17 |
| 2485 , 3.470 | all cis-5, 8, 11, 14-Eicosatetraenoic acid, methyl ester (Arachidonic) | 20:4 ω6, cis | | Not Found | 43.95 |
| 2495 , 3.850 | all cis-5, 8, 11, 14, 17-Eicosapentaenoic acid, methyl ester (EPA) | 20:5 ω3, cis | | Not Found | 0.18 |
| 2530 , 3.200 | all cis-11, 14, 17-Eicosatrienoic acid, methyl ester | 20:3 ω3, cis | | Not Found | 0.24 |
| 2580 , 2.930 | cis, cis-11, 14- Eicosadienoic acid, methyl ester | 20:2 ω6, cis | | Not Found | 1.76 |
| 2595 , 2.630 | cis-11-Eicosenoic acid, methyl ester | 20:1 ω9, cis | 55 | 0.62 | 2.26 |
| 2595 , 3.250 | all cis-8,11,14-Eicosatrienoic acid, methyl ester | 20:3 ω6, cis | | Not Found | Not Found |
| 2655 , 2.350 | Eicosanoic acid, methyl ester (Arachidic) | 20:0 | 74 | 1.17 | 0.16 |
| 2890 , 2.410 | Heneicosanoic acid, methyl ester | 21:0 | 74 | 0.05 | Not Found |
| 2930 , 4.360 | all cis-4, 7, 10, 13, 16, 19-Docosahexaenoic acid, all cis, methyl ester (DHA) | 22:6 ω3, cis | | Not Found | 23.25 |
| 3045 , 3.150 | cis, cis-13, 16- Docosadienoic acid, methyl ester | 22:2 ω6, cis | | Not Found | Not Found |
| 3060 , 2.850 | cis-13-Docosenoic acid, cis, methyl ester (Erucic) | 22:1 ω9, cis | | Not Found | Not Found |
| 3115 , 2.570 | Docosanoic acid, methyl ester (Behenic) | 22:0 | 74 | 0.24 | Not Found |
| 3340 , 2.650 | Tricosanoic acid, methyl ester | 23:0 | | Not Found | Not Found |
| 3500 , 3.090 | cis-15-Tetracosenoic acid, methyl ester (Nervonic) | 24:1 ω9, cis | | Not Found | Not Found |
| 3555 , 2.780 | Tetracosanoic acid, methyl ester (Lignoceric) | 24:0 | 74 | 0.21 | Not Found |

The structure of individual FAMEs can be suggested based on their position in the contour plot, without additional MS information or comparison with a standard. For this reason, the method could be applied for routine analysis using GCxGC-FID.

Quantification of fatty acids in two real-life samples was performed using the automated calibration function of ChromaTOF software.

## 5. Acknowledgements

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