

AUTOMATIC DERIVATIZATION COUPLED WITH GAS CHROMATOGRAPHY-CHEMICAL IONIZATION MASS SPECTROMETRY FOR THE ANALYSIS OF FATTY ACIDS IN FOOD SAMPLES

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INTRODUCTION

The analysis of Fatty Acid Methyl Esters (FAMES), which characterizes the lipid fraction in food, constitutes a main application in food quality control. Most edible fats and oils contain primarily linear saturated fatty acids, but branched and unsaturated ones can also occur. Moreover, the determination of double bond(s) position and geometric configuration *cis/trans* is also important. In fact, the US FDA recently amended its regulations to include the amount of *trans* fatty acids in the Nutrition Facts panel of food due their adverse health effects.

Fatty acids are usually analysed by GC after methylation, which involves the optimisation of both analysis time to maximize throughput and selectivity of GC method to ensure resolution and reliable quantification of *cis/trans* pairs.

OVERVIEW

AIM: To establish a procedure for the determination of fatty acids in different matrices using automatic derivatization on KONIK ROBOKROM Autosampler coupled with KONIK HRGC 4000B and KONIK MS Q12 quadrupole instrument working in both modes, electronic impact (EI) and positive chemical ionization (PCI).

METHODS: GC-MS (EI+, PCI)

RESULTS: KONIK Robokrom Autosampler coupled with KONIK HRGC 4000B and MS Q12 instrument has been successfully applied as a fast method for the analysis of fatty acids in food matrices using EI and especially PCI for a better identification of *cis/trans* isomers.

EXPERIMENTAL

Samples: Oil and fat samples as well as food samples were obtained from Barcelona market, Spain.

Standards: A 37-component fatty acid methyl ester (FAMES) mixture (Supelco 47895-U) was used. The mixture was purchased as 10 mg/ml of the FAME reference standard mix in dichloromethane, containing C4 to C24 FAMES (2 to 6% relative concentration). Secondary standards were prepared by dilution of this mixture in hexane.

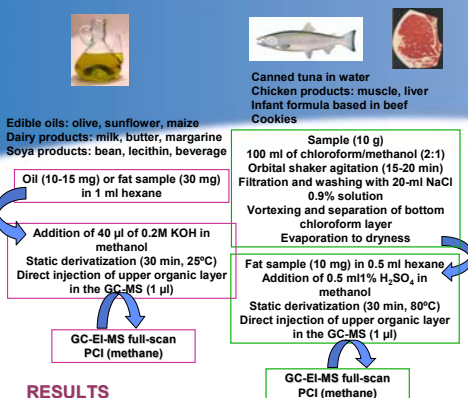
To optimize cold basic transesterification procedure, olive oil was used as representative. For comparison, acid hydrolysis was also employed for animal food.



Autosampler-GC-MS Conditions

GC Equipment:	KONIK HRGC 4000B
Column:	TR-CN100, 60 m, 0.25 mm, 0.20 µm
Carrier:	He; constant flow 1 ml/min
Injector:	250°C, injection mode: splitless/split; splitless time: 2 min (CI) and split (EI); split ratio: 1:50
Oven:	40°C (2 min); 10°C/min; 150; 3°C/min; 240°C (10 min)
MS Equipment:	KONIK MS Q12 quadrupole
Ionization mode:	EI+ and PCI
Energy:	70 eV
Source T:	120°C for both EI and PCI
Reagent gas:	Methane at 1 ml/min
Transfer line T:	250°C
Scan range:	m/z 40-400 (EI+); m/z 40-430 (PCI)
Scan rate:	3 ms
Injector GC Equipment:	KONIK ROBOKROM AUTOSAMPLER
Tray:	105 vials of 2 ml; 1 ml of sample/standard; room temperature
Syringe type:	Hamilton 50 µl
Derivat. T:	25°C (cold basic transesterification); 80°C (acid methylation)
Derivat. Vol:	2 M KOH in methanol or 1% H ₂ SO ₄ in methanol
Derivat. Type:	40 µl / 500 µl (10 x 50 µl)
Deriv. time:	30 min
Agitation:	Static

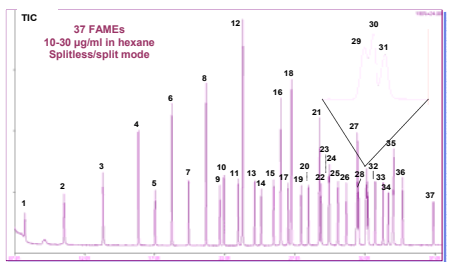
Sample Analysis Methodology



RESULTS

a- CHROMATOGRAPHIC SEPARATION

FAMES were well separated using a 100% cyanopropyl polysiloxane column, which is excellent for resolving FAMES (*cis-trans*-isomers), derivatised sugars, PCBs and dioxins.



b- GC-MS EI/PCI FAMES SPECTRA

Peak #	CI of FAME (m/z)				Peak #	CI of FAME (m/z)				
	Compound	EI Ion*	[M+1] ⁺	[M+29] ⁺		Compound	EI Ion*	[M+1] ⁺	[M+29] ⁺	
1	C6:0	74.03	103	71	21	C18:2n-6	327	323	263	
2	C8:0	74.03	131	89	22	C18:2n-6	327	323	263	
3	C10:0	74.07	189	127	23	C18:2n-6	327	323	263	
4	C12:0	74.07	187	165	23	C18:3n-3	327	323	263	
5	C14:0	74.07	291	229	24	C20:1	355	351	289	
6	C16:0	74.07	218	243	181	26	C22:3	347	343	281
7	C18:0	74.07	229	287	197	26	C22:3	347	343	281
8	C18:1n-7	74.07	218	243	181	26	C22:3	347	343	281
9	C18:1n-9	74.07	218	243	181	26	C22:3	347	343	281
10	C18:2n-6	74.07	287	285	223	29	C26:3	375	371	309
11	C18:2n-6	74.07	287	285	223	31	C26:3	375	371	309
12	C18:2n-6	74.07	287	285	223	31	C26:3	375	371	309
13	C18:2n-6	74.07	287	285	223	31	C26:3	375	371	309
14	C17:0	74.07	287	285	223	31	C26:3	375	371	309
15	C17:1	74.07	287	285	223	31	C26:3	375	371	309
16	C17:2	74.07	287	285	223	31	C26:3	375	371	309
17	C17:3	74.07	287	285	223	31	C26:3	375	371	309
18	C18:1n-9	74.07	287	285	223	31	C26:3	375	371	309
19	C18:2n-6	74.07	287	285	223	31	C26:3	375	371	309

* Most abundant ions; b not detected

GC-MS analyses in EI mode result in strong fragmentation and rearrangement of FAMES that often do not yield a molecular ion [M]⁺ peak representing the molecular weight of the FAME, particularly as the MW increases. EI ions at m/z 74 and 87 are attributed to the McLafferty rearrangement, especially in case of saturated acids.

EI mass spectra of unsaturated fatty acids differ from those of their saturated analogues, and they also vary a little according to degree of unsaturation. For dienes and trienes, the molecular ion is more pronounced, while those representing losses of 32, 74 and 116 amu are less so.

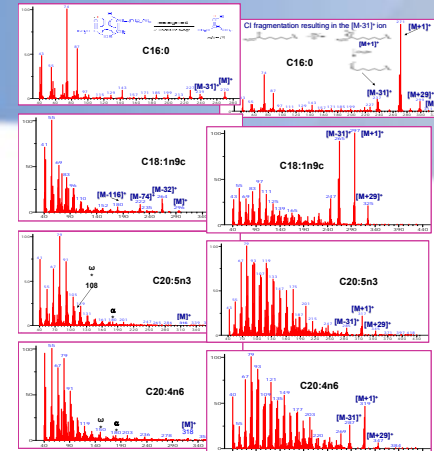
Methyl esters of different fatty acids of (n-3) family give a characteristic fragment at m/z = 108, while those of (n-6) series give a prominent ion at m/z = 150. These ions represent fragments from the terminal region of the molecule.

The most important contribution of PCI spectra was unequivocal establishment of MW of FAMES. This was identified from the protonated molecular ion [M+1]⁺. Moreover, all CI spectra contain [M-1]⁺ ions formed from a hybrid transfer from the original FAME or the loss of H₂ from an [M+1]⁺ ion.

Ions at [M+29]⁺ or even at [M+41]⁺ in PCI spectra were formed by adding C2H5⁺ and C3H5⁺ from methane, respectively, to the molecule and help to confirm the assignment of [M-1]⁺ ion in the spectra.

Ions at [M-31]⁺ and [M-33]⁺ represent losses of CH₃COH from [M+1]⁺ ion (saturated FAMES) and [M-1]⁺ ion (unsaturated and monounsaturated FAMES), respectively.

EI vs. PCI FAMES SPECTRA



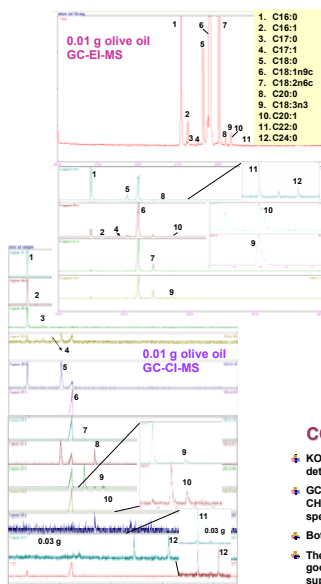
c- GC-MS QUALITY PARAMETERS

Parameter	GC-EI-MS	GC-PCI-MS
LOD* (ng injected):	0.008- 0.15	0.15 - 6.0
Precision RSD(n=10):	2.6 - 5.1	3.0 - 6.3
Linearity†:	0.9954-0.9999	0.9942- 0.9999

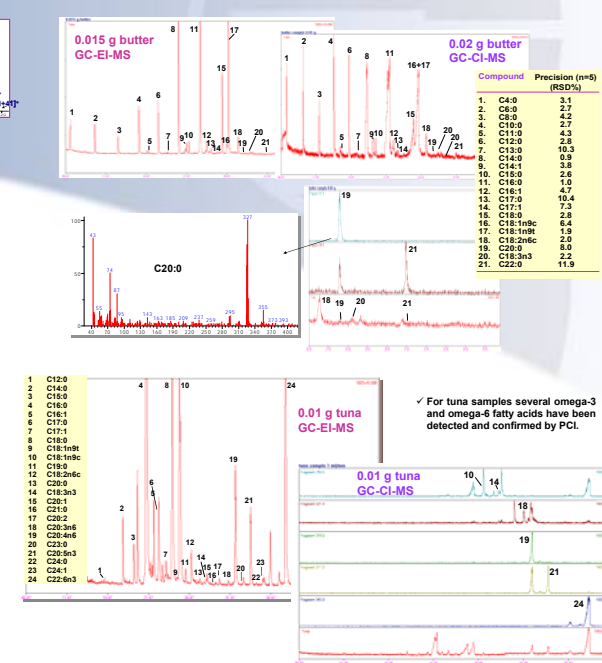
* Calculated as S/N = 3 and splitless/split mode; † n=10; concentration level 2-6 ppm
 ‡ Corr. coef. (r₂); linearly studied from 0.4 ppm to 200 ppm

For CI, the [M+1]⁺ ion was used to determine LODs. However, the sum of two ions, such as [M+1]⁺ and [M+29]⁺ or [M+1]⁺ + [M-31]⁺ can be used if necessary to improve LOD values.

d- SAMPLE ANALYSIS BY GC-MS



d- SAMPLE ANALYSIS BY GC-MS



Fatty acid composition of oils and fats of vegetable sources, dairy products and in foods of animal origin (n=3) (expressed as percentage mass fraction of total fatty acids; RSD% between clauses)

Compound	Olive oil	Sunflower oil	Maize oil	Soya bean	Soya + cereals beverage	Soya lecithin	Cookies	Milk (cow)	Butter	Margarine	Tuna	Chicken skin	Liver (chicken)	Infant formula
C4:0	2.9 (4.1)	2.8 (3.2)	0.01 (6.1)
C6:0	2.3 (3.8)	2.2 (3.2)	0.01 (6.1)
C8:0	1.7 (4.2)	1.5 (4.9)	1.1 (5.3)	0.05 (8.2)
C10:0	3.5 (2.8)	3.1 (1.6)	1.1 (6.2)	0.30 (16.8)
C12:0	0.02 (4.0)	0.06 (5.3)	0.26 (2.3)
C14:0	0.15 (5.0)	0.10 (6.3)
C16:0	23.7 (6.3)	4.2 (2.8)	4.2 (2.8)	10.3 (5.2)	0.05 (7.2)	0.04 (9.6)	...
C18:0	0.03 (6.3)	0.16 (4.2)	0.16 (3.7)	0.01 (5.1)
C18:1n-7	1.2 (8.2)	0.97 (3.8)	0.35 (3.1)	2.2 (6.3)
C18:1n-9	0.11 (5.6)	0.72 (4.8)	0.20 (2.9)	0.15 (3.3)	0.07 (4.6)
C18:2n-6	0.33 (3.2)	0.07 (1.2)	0.05 (1.2)	0.39 (3.8)
C18:3n-3	0.64 (5.2)	0.65 (7.7)	0.81 (10.0)	16.5 (3.5)	15.0 (2.8)	15.0 (2.8)	...
C20:1	1.7 (3.9)	1.2 (3.0)	0.04 (3.7)	0.82 (6.3)	0.10 (7.1)	0.03 (2.8)	0.25 (8.2)
C20:5n3	0.22 (9.8)
C20:4n6	0.16 (2.8)	0.09 (0.9)	0.16 (2.8)	0.26 (2.6)	0.22 (6.0)
C22:3	0.04 (5.2)	0.05 (7.7)	0.01 (10.0)	0.11 (5.6)	0.72 (4.8)	0.20 (2.9)	0.15 (3.3)
C22:6	0.33 (3.2)	0.07 (1.2)	0.05 (1.2)	0.39 (3.8)
C24:1	1.7 (3.9)	1.2 (3.0)	0.04 (3.7)	0.82 (6.3)	0.10 (7.1)	0.03 (2.8)	0.25 (8.2)
C24:2n-6	0.22 (9.8)
C24:3n-3	0.16 (2.8)	0.09 (0.9)	0.16 (2.8)	0.26 (2.6)	0.22 (6.0)
C26:3	0.04 (5.2)	0.05 (7.7)	0.01 (10.0)	0.11 (5.6)	0.72 (4.8)	0.20 (2.9)	0.15 (3.3)
C26:6	0.33 (3.2)	0.07 (1.2)	0.05 (1.2)	0.39 (3.8)
C28:1	1.7 (3.9)	1.2 (3.0)	0.04 (3.7)	0.82 (6.3)	0.10 (7.1)	0.03 (2.8)	0.25 (8.2)
C28:2n-6	0.22 (9.8)
C28:3n-3	0.16 (2.8)	0.09 (0.9)	0.16 (2.8)	0.26 (2.6)	0.22 (6.0)
C30:1	0.04 (5.2)	0.05 (7.7)	0.01 (10.0)	0.11 (5.6)	0.72 (4.8)	0.20 (2.9)	0.15 (3.3)
C30:2n-6	0.33 (3.2)	0.07 (1.2)	0.05 (1.2)	0.39 (3.8)
C30:3n-3	1.7 (3.9)	1.2 (3.0)	0.04 (3.7)	0.82 (6.3)	0.10 (7.1)	0.03 (2.8)	0.25 (8.2)
C32:1	0.22 (9.8)
C32:2n-6	0.16 (2.8)	0.09 (0.9)	0.16 (2.8)	0.26 (2.6)	0.22 (6.0)
C32:3n-3	0.04 (5.2)	0.05 (7.7)	0.01 (10.0)	0.11 (5.6)	0.72 (4.8)	0.20 (2.9)	0.15 (3.3)
C34:1	0.33 (3.2)	0.07 (1.2)	0.05 (1.2)	0.39 (3.8)
C34:2n-6	1.7 (3.9)	1.2 (3.0)	0.04 (3.7)	0.82 (6.3)	0.10 (7.1)	0.03 (2.8)	0.25 (8.2)
C34:3n-3	0.22 (9.8)
C36:1	0.16 (2.8)	0.09 (0.9)	0.16 (2.8)	0.26 (2.6)	0.22 (6.0)
C36:2n-6	0.04 (5.2)	0.05 (7.7)	0.01 (10.0)	0.11 (5.6)	0.72 (4.8)	0.20 (2.9)	0.15 (3.3)
C36:3n-3	0.33 (3.2)	0.07 (1.2)	0.05 (1.2)	0.39 (3.8)
C38:1	1.7 (3.9)	1.2 (3.0)	0.04 (3.7)	0.82 (6.3)	0.10 (7.1)	0.03 (2.8)	0.25 (8.2)
C38:2n-6	0.22 (9.8)
C38:3n-3	0.16 (2.						