Determination of Fatty Acid Methyl Esters (FAMEs) in Milk Matrix Using an Agilent 5977E GC/MS

Application Note

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Abstract

This application note describes a method for the analysis of fatty acid methyl esters (FAMEs) on a DB-Wax column using an Agilent 5977E GC/MSD. The method works well with a good calibration response; the instrument detection limit (IDL) for each of the tested analytes can reach 0.01 ppm.

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Introduction

The analysis of FAMEs is used for the characterization of the lipid fraction in foods, and is one of the most important applications in food analysis. Lipids mainly consist of triglycerides, which are esters of one glycerol molecule and three fatty acids. Most edible fats and oils are composed primarily of 12- to 20-carbon fatty acids (lauric acid (dodecanoic acid) to arachidic acid (eicosanoic acid)). In addition to linear saturated fatty acids, branched, mono-unsaturated, di-unsaturated, and higher unsaturated fatty acids occur.

The method in this application note uses a DB-Wax column that separates FAMEs from C4 (butyric acid) to C24 (lignoceric acid) according to carbon number and unsaturation. On this column, no separation of *cis*- and *trans*- isomers was obtained, and for complex mixtures (such as fish oils), some FAMEs were not resolved. However, the separation of FAMEs on polyethylene glycol columns is widely used and can be applied to the characterization of classic samples such as vegetable oils (corn oil, maize oil, olive oil, soybean oil, and so on).

Experiment

Reagents and chemicals

For the standards, a mixture of 100 mg of 37 FAMEs, individually varied between 2–6 mg, were dissolved in hexane with internal standard biphenyl. The whole milk was purchased from a local supermarket.

Table 1. Sample Parameters

Sample	Form	Appearance
37 FAMEs mixture	100 mg white liquid	–
Internal standard biphenyl	–	White solid
Milk	2 L	White liquid

Equipment and materials

This method was developed on the Agilent 5977E GC/MS. An Agilent DB-WAX 30 m × 250 µm, 0.5 µm column was used (p/n 122-7033). Table 2 lists the conditions.

Standard preparation

A 50 mg standards mix was dissolved in 50 mL of hexane. The stock solution was then diluted to 500, 200, 100, 50, 20, 10, 5, 2, and 1 ppm (total concentration) solution with spiked biphenyl (100 ppm).

Sample preparation

The milk sample was purchased from local store. A 0.5 mL amount of milk sample was placed in a 20-mL vial with 10 mL of hexane and 1 mL of sodium methoxide (5.4 M) in methanol solution. After strong vortexing for 1 minute, the clear hexane layer was extracted for GC/MS analysis.

Table 2. GC/MSD Instrument Parameters

Instrument	Agilent 5977E GC/MS
Oven	
Oven profile	50 °C for 1 minute, then 25 °C/min to 200 °C, then 3 °C/min to 230 °C for 23 minutes
Run time	40 minutes
Equilibration time	0.5 minutes
Inlet	
Injector type	Pulsed split
Temperature	250 °C
Injection volume	3 µL
Split ratio	5:1
Column head pressure	40 psi for 0.4 minutes, constant pressure at 20 psi
Carrier gas	He
MS	
Transfer line temperature	250 °C
Mode	Scan and SIM
Source temperature	230 °C
Quadrupole temperature	150 °C
Mass range *m/z*	46–500 u

Results and Discussion

Figure 1 shows a typical chromatogram for the analysis of the FAME reference standard. Table 3 lists the retention time and SIM ions of the targets. A good separation is obtained, except for the following compounds: *cis* and *trans* 18:1 coelute at 14.20 minutes, *cis* and *trans* 18:2 coelute at 15.032 minutes, 20:3 *n*-6 and 21:0 coelute at 19.55 minutes, and 22:6 and 24:1 coelute at 31.814 minutes. However, this separation is sufficient for fat characterization.

Figure 2 is a SIM chromatogram of 37 compounds from 30 to approximately 60 ppb.

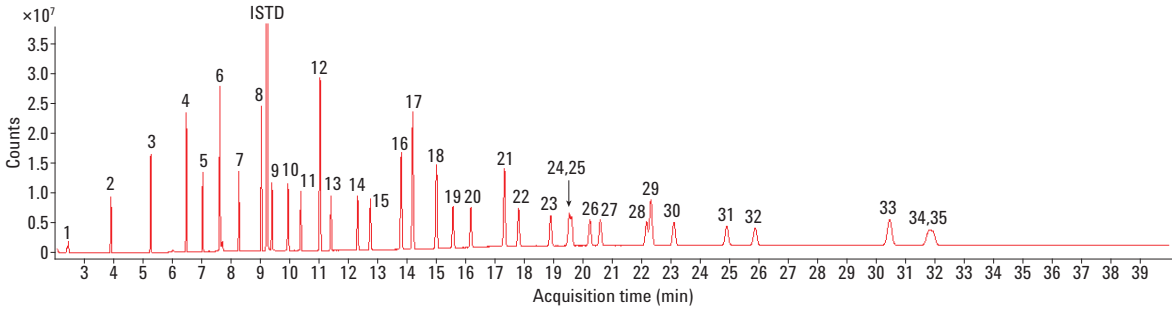

Figure 1. Total ion chromatogram of full scan for the 37 compounds; each of peak 17 and 18 concludes 2 compounds. The concentration is from 100 to 300 ppm.

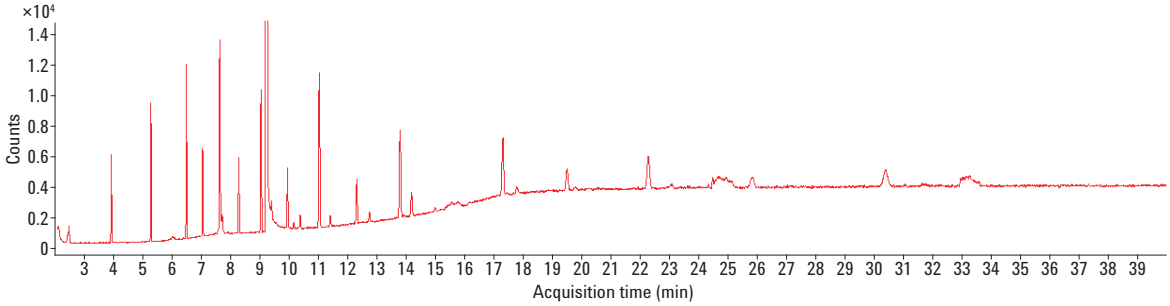

Figure 2. SIM chromatogram one of monitored mass (m/z 74). Component concentration 30–60 ppb.

Table 3. Compound List

		FAME	Retention time (min)	SIM ions, *m/z*	
1	C4:0	Butyric	2.464	74.1	87.1
2	C6:0	Caproic	3.934	74	87.1
3	C8:0	Caprylic	5.294	74	87.1
4	C10:0	Capric	6.509	74	87.1
5	C11:0	Undecanoic	7.07	74.1	87.1
6	C12:0	Lauric	7.651	74	87
7	C13:0	Tridecanoic	8.302	74	87.1
8	C14:0	Myristic	9.067	74	87.1
	ISTD	Biphenyl	9.251	74	–
9	C14:1	Myristoleic	9.427	55.1	74.1
10	C15:0	Pentadecaoic	9.976	74	87
11	C15:1	*cis*-10-Petadecenoic	10.41	55.1	74.1
12	C16:0	Palmitic	11.064	74	87.1
13	C16:1	Palmitoleic	11.436	55.1	74.1
14	C17:0	Heptadecanoic	12.346	74	87
15	C17:1	*cis*-10-Heptadecenoic	12.779	55.1	69.1
16	C18:0	Stearic	13.829	74.1	87.1
17	C18:1 c+t	Elaidic, Oleic	14.2	55.1	69.1
18	C18:2 c+t	Linoleaidic, Linoleic	15.032	67.1	81.1
19	C18:3 n-6	γ-Linolenic	15.602	79.1	67.1
20	C18:3 n-3	Linolenic	16.21	79.1	67.1
21	C20:0	Arachidic	17.338	74	87
22	C20:1	*cis*-11-Eicosenoic	17.83	55.1	69.1
23	C20:2	*cis*-11,14-Eicosadienoic	18.924	67.1	81.1
24	C21:0	Heneicosanoic	19.55	74	87
25	C20:3 n-6	*cis*-8,11,14-Eicosatrienoic	19.631	79.1	67.1
26	C20:4	Arachidonic	20.271	79.1	91.1
27	C20:3 n-3	*cis*-11,14,17-Eicosatrienoic	20.618	79.1	95.1
28	C20:5	*cis*-5,8,11,14,17-Eicosapentaenoic	22.221	79.1	99.1
29	C22:0	Behenic	22.335	74	87.1
30	C22:1	Erucic	23.129	87.1	73.1
31	C22:2	*cis*-13,16-Docosadienoic	24.939	81.1	67.1
32	C23:0	Tricosanoic	25.893	87.1	74.1
33	C24:0	Lignoceric	30.472	74	87
34	C24:1	Nervonic	31.814	55.1	69.1
35	C22:6	*cis*-4,7,10,13,16,19-Docosahexaenoic	31.968	79.1	91

Calibration curve coefficients and milk contents are listed in Table 4. A milk extract SIM chromatogram is shown in Figure 3.

Table 4. Calibration Data and Quantitation Results in Milk

Analytes	FAME	R^2	Lowest linear concentration (ppm)	Concentration in hexane (ppm)	Concentration in milk (g/L)
C4:0	Butyric	0.9996	0.4	146	2.92
C6:0	Caproic	0.9998	0.4	70	1.4
C8:0	Caprylic	0.9998	0.4	40	0.8
C10:0	Capric	0.9992	0.8	72	1.44
C11:0	Undecanoic	0.9999	0.2	1	0.02
C12:0	Lauric	0.9994	0.8	65	1.3
C13:0	Tridecanoic	0.9994	0.2	1	0.02
C14:0	Myristic	0.9998	0.8	107	2.14
ISTD	Biphenyl	–	–	–	–
C14:1	Myristoleic	0.9988	0.2	14	0.28
C15:0	Pentadecaoic	0.9999	1	8	0.16
C15:1	*cis*-10-Petadecenoic	0.9994	0.2	4	0.08
C16:0	Palmitic	0.9998	0.6	163	3.26
C16:1	Palmitoleic	0.9996	0.2	14	0.28
C17:0	Heptadecanoic	0.9995	0.2	2	0.04
C17:1	*cis*-10-Heptadecenoic	0.9996	0.2	–	–
C18:0	Stearic	0.9998	0.4	37	0.74
C18:1 c+t	Elaidic, Oleic	0.9998	0.6	105	2.1
C18:2 c+t	Linoleaidic, Linoleic	0.9997	0.4	13	0.26
C18:3 n-6	γ-Linolenic	0.9996	0.2	1	0.02
C18:3 n-3	Linolenic	0.9996	0.2	4	0.08
C20:0	Arachidic	0.9997	0.4	–	
C20:1	*cis*-11-Eicosenoic	0.9996	0.02	–	
C20:2	*cis*-11,14-Eicosadienoic	0.9995	0.02	–	
C21:0	Heneicosanoic	0.9995	0.02	–	
C20:3 n-6	*cis*-8,11,14-Eicosatrienoic	0.9995	0.02	–	
C20:4	Arachidonic	0.9996	0.02	–	
C20:3 n-3	*cis*-11,14,17-Eicosatrienoic	0.9994	0.02	–	
C20:5	*cis*-5,8,11,14,17-Eicosapentaenoic	0.9996	0.02	–	
C22:0	Behenic	0.9996	0.04	–	
C22:1	Erucic	1	0.2	–	
C22:2	*cis*-13,16-Docosadienoic	0.9997	0.2	–	
C23:0	Tricosanoic	0.9998	0.4	–	
C24:0	Lignoceric	0.9997	0.4	–	
C24:1	Nervonic	0.9996	0.2	–	
C22:6	*cis*-4,7,10,13,16,19-Docosahexaenoic	0.9998	0.2	–	

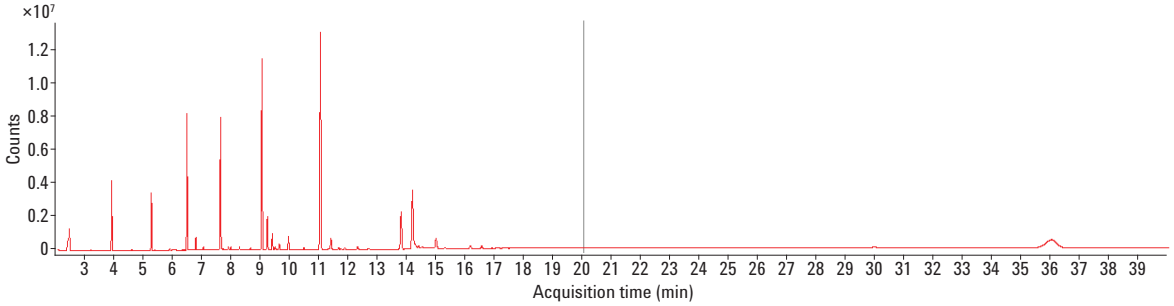

Figure 3. SIM chromatogram of milk extract.

Instrument detection limit (IDL)

The sample with lowest concentration (0.01 ppm with 1 ppm internal standard) was injected 10 times to determine the IDL. The results were based on a 1 µL injection with 10 ng of material in pulsed splitless mode.

Table 5. IDL Results

Analyte	FAME	IDL (ppm)	Analyte	FAME	IDL (ppm)
C4:0	Butyric	0.1155	C18:3 n-6	γ-Linolenic	0.0504
C6:0	Caproic	0.1012	C18:3 n-3	Linolenic	0.0496
C8:0	Caprylic	0.1001	C20:0	Arachidic	0.0942
C10:0	Capric	0.0998	C20:1	*cis*-11-Eicosenoic	0.0472
C11:0	Undecanoic	0.0500	C20:2	*cis*-11,14-Eicosadienoic	0.0455
C12:0	Lauric	0.0997	C21:0	Heneicosanoic	0.0493
C13:0	Tridecanoic	0.0505	C20:3 n-6	*cis*-8,11,14-Eicosatrienoic	0.0490
C14:0	Myristic	0.1010	C20:4	Arachidonic	0.0499
C14:1	Myristoleic	0.0528	C20:3 n-3	*cis*-11,14,17-Eicosatrienoic	0.0500
C15:0	Pentadecaoic	0.0507	C20:5	*cis*-5,8,11,14,17-Eicosapentaenoic	0.0500
C15:1	*cis*-10-Petadecenoic	0.0522	C22:0	Behenic	0.1003
C16:0	Palmitic	0.1519	C22:1	Erucic	0.1241
C16:1	Palmitoleic	0.0530	C22:2	*cis*-13,16-Docosadienoic	0.0518
C17:0	Heptadecanoic	0.0509	C23:0	Tricosanoic	0.0900
C17:1	*cis*-10-Heptadecenoic	0.0497	C24:0	Lignoceric	0.0985
C18:0	Stearic	0.0967	C24:1	Nervonic	0.0770
C18:1 c+t	Elaidic, Oleic	0.1524	C22:6	*cis*-4,7,10,13,16,19-Docosahexaenoic	0.0527
C18:2 c+t	Linoleaidic, Linoleic	0.0932			

Conclusion

The Agilent 5977E GC/MS shows good results for FAMEs in linearity, IDL, and separation, and is suitable for the detection of FAMEs.

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Printed in the USA
September 15, 2014
5991-4867EN

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