Analysis of Fatty Acid Methyl Esters by Agilent 5975T LTM GCMS

Application Note

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Abstract

The analysis of fatty acid methyl esters (FAMES) is a very important application in food analysis. They are often detected in lab with routine GCMS, Agilent 5975T transportable GC/MS can run the application in the field and delivers the same reliability, high performance and quality results as our lab Agilent 5975 Series GC/MSD system, it is ideally suited for out-of-lab analysis when fast and timely response is required.

In this method, the analysis of FAMES is performed on a DB-Wax column using an Agilent 5975T LTM GC/MSD with a Thermal Separation Probe (TSP). Retention Time Locked Methods and Retention Time Database is applied for the analysis. Retention time locking allows easy peak identification, easy exchange of data between instruments, and avoids the need to modify the retention times in calibration tables after column maintenance or column change.

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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 largely 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 can occur. Table 1 shows an overview of the most important fatty acids and their common abbreviations.

Although the free fatty acids can be analyzed directly on polar stationary phases (such as a FFAP column), more robust and reproducible chromatographic data are obtained if the fatty acids are derivatized to the FAMEs. Several methods are available for derivatization, which requires hydrolysis of the glycerides and methylation of the resulting fatty acids. These easy-to-use methods do not require expensive reagents or equipment.

Table 1. Fatty Acids, Common Names and Abbreviation Used

Fatty acid	Common name	Simplified abbreviation[1]	Abbreviation specifying *cis* and *trans* bonds[1]
Butanoic acid	Butyric acid	4:0	4:0
Decanoic acid	Caproic acid	10:0	10:0
Dodecanoic acid	Lauric acid	12:0	12:0
Tetradecanoic acid	Myristic acid	14:0	14:0
Hexadecanoic acid	Palmitic acid	16:0	16:0
Hexadecenoic acid	Palmitoleic acid	16:1 n-7	9*c*-16:1
Octadecanoic acid	Stearic acid	18:0	18:0
cis-9-Octadecenoic acid	Oleic acid	18:1 n-9	9*c*-18:1
trans-9-Octadecenoic acid	Elaidic acid	*t*18:1 n-9	9*t*-18:1
all *cis*-9,12-Octadecadienoic acid	Linoleic acid	18:2 n-6	9*c*12*c*-18:2
ll *trans*-9,12-Octadecadienoic acid	Linolelaidic acid	*t*18:2 n-6	9*t*12*t*-18:2
all *cis*-9,12,15-Octadecatrienoic acid	*alpha*-Linolenic acid	18:3 n-3	9*c*12*c*15*c*-18:3
all *cis* -6,9,12-Octadecatrienoic acid	*gamma*-Linolenic acid	18:3 n-6	6*c*9*c*12*c*-18:3
Eicosanoic acid	Arachidic acid	20:0	20:0
cis-11-Eicosenoic acid		20:1 n-9	11*c*-20:1
all *cis* -11,14-Eicosadienoic acid		20:2 n-6	11*c*14*c*-20:2
all *cis* -11,14,17-Eicosatrienoic acid		20:3 n-3	11*c*14*c*17*c*-20:3
all *cis* -8,11,14-Eicosatrienoic acid	Dihomogammalinolenic acid	20:3 n-6	8*c*11*c*14*c*-20:3
all *cis* -5,8,11,14-Eicosatetraenoic acid	Arachidonic acid	20:4 n-6	5*c*8*c*11*c*14*c*-20:4
all *cis* 5,8,11,14,17-Eicosapentaenoic acid	EPA	20:5 n-3	5*c*8*c*11*c*14*c*17*c*-20:5
Docosanoic acid	Behenic acid	22:0	22:0
cis -13-Docosenoic acid	Erucic acid	22:1 n-9	13*c*-22:1
all *cis* -7,10,13,16-Docosatetraenoic acid		22:4 n-6	7*c*10*c*13*c*16*c*-22:4
all *cis* 4,7,10,13,16,19-Docosahexaenoic acid	DHA	22:6 n-3	4*c*7*c*10*c*13*c*16c19*c*-22:6

[1]Several different versions of fatty acid nomenclature and structural abbreviation have been used in the past. For discussions of past and currently-accepted nomenclature, the following web sites are recommended:
http://www.ajcn.org/misc/lipid.shtml
http://www.chem.qmul.ac.uk/iupac/lipid/
http://www.aocs.org/member/division/analytic/fanames.htm
http://www.cyberlipid.org/index.htm

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 is obtained, and for complex mixtures (such as fish oils), some FAMEs are not resolved. However, the separation of FAMEs on polyethylene glycol columns is widely used and can be applied to the characterization of classical samples such as vegetable oils (corn oil, maize oil, olive oil, soybean oil, and so on). For certain applications, animal fats can also be analyzed using an Agilent DB-Wax column. An important application, for instance, is the analysis of butyric acid in milk fat. The concentration of butyric acid in milk is an important indicator of its quality. This determination is very important in milk and dairy analysis and in the analysis of chocolate products. Because the low volatile food matrix may contaminate the system and interfere the results, we use TSP as an injection tool to increase the system uptime and save the operation cost. Additionally, mobile lab can provide faster and more economic detection for FAMEs detection.

The method used in this application note is retention time locked by using methyl stearate as the locking compound. Because RTL reproduces retention times so accurately, FAME identification can be done based on absolute retention times. It is unnecessary to have all of the FAME standards available because peak identification is possible using the Agilent published retention time database. An additional benefit of RTL is that retention times in the calibration table remain unchanged even after column maintenance or column change (after re-locking the method).

Experimental

Samples

Reference standards of FAMEs can be obtained from different sources as solutions or as neat compounds. For analysis, the standards are typically dissolved in hexane at a 0.01 to 0.1% (w/v) concentration. For method and instrument check-out, a 37-component mixture (Supelco number 18919) was used. The mixture was purchased as a 100-mg neat mixture, containing C4 to C24 FAMEs (2 to 4% relative concentration). The whole sample was diluted in 10 mL of hexane (final concentration = 0.2 to 0.4 mg/mL per FAME).

Sample preparation

Weigh a 10-mg sample in a 2-mL test tube (with screw cap) or a reaction vial. Dissolve the sample in 1 mL of hexane. Add 10 µL of 2N potassium hydroxide in methanol (11.2 g in 100 mL). Close the tube or vial, and vortex for 30 seconds. Centrifuge . Transfer 1 µL clear supernatant to TSP microvial.

Analytical Conditions

The analyses were performed on a 5975T GC/MSD system. Automated split injection was performed using TSP. Table 2 summarizes the instrumental configuration and analytical conditions. Methyl stearate was used as the locking standard. The retention time for methyl stearate was locked at 14.000 minutes.

Table 2. Parameters of the Instrument

Instrumentation	**Agilent 5975T LTM GCMS**
Column	DB-wax LTM module 30 m × 0.25 mm × 0.25 µm (Customized 100-2000 LTM)
Guard column	0.5 m column with the same phase as the analytical column, connected to the injector
Experimental conditions	
Inlet temperature	250 °C
Injection mode	TSP
Injection mode	split, 50:1
Carrier gas	helium
Constant pressure mode	Methyl stearate is retention time locked to 14.000 minutes (pressure approximately 55 kPa at 50 °C, 36 cm/sat 50 °C)
LTM Oven	50 °C 1 minute to 25 °C/min to 200 °C, 3 °C/min to 230 °C, 18 minutes
Isothermal temperature	250 °C
MSD interface	250 °C
Ion source	230 °C
Quad. temperature	150 °C
Ionization mode	EI
Scan mode	full scan, m/z 40-500
EMV mode	Gain factor
Gain factor	5.00
Resulting EM voltage	1,400 V
Solvent delay	2.0 minutes

Results and Discussion

Figure 1 shows a typical chromatogram for the analysis of the FAME reference standard. A good separation is obtained, except for the following compounds: *cis* and *trans* 18:1 co-elute at 14.38 minutes, *cis* and *trans* 18:2 co-elute at 15.13 minutes, 20:3 n-6 and 21:0 co-elute at 19.44 minutes, and 22:6 and 24:1 co-elute at 30.73 minutes. However, this separation is sufficient for most classical oil and fat characterization. Butyric acid elutes at 2.85 minutes and can be determined in milk fat using the same method.

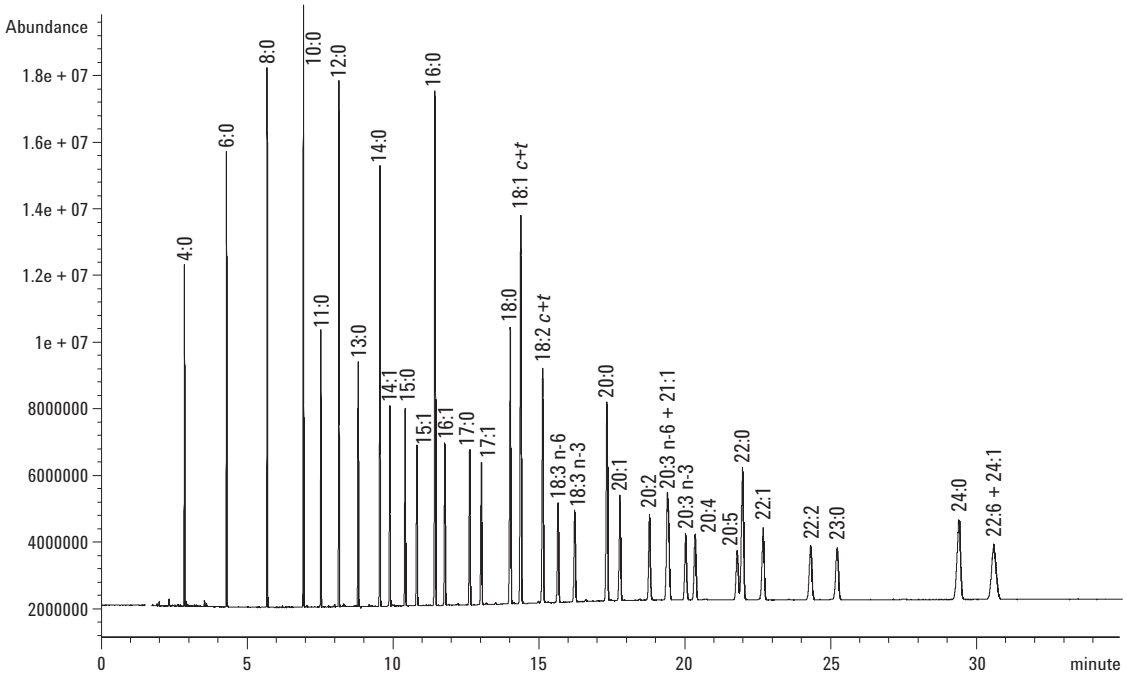

Figure 1 Analysis of FAMEs standard mixture on a 30-m × 0.25-mm id 0.25-µm DB-Wax column

The retention time locked method on the DB-Wax column was applied to the analysis of two certified reference samples (CRM 162, a soy-maize blend oil and CRM 164, a milk fat). The peaks were automatically identified using the RTL FAMEs retention time database. Butyric acid elutes at 2.85 minutes and is easily detected. Very good reproducibility is obtained. The standard deviation of the relative areas is smaller than 1% in all cases. Also, the correspondence between the measured fatty acid composition and the certified values is good. The quantitative results are summarized in Tables 3 and 4.

Table 3. Quantitative Data of Detected Components for CRM 162

Fatty acid	Measured concentration (g/100 g)	Standard deviation	Certified concentration (g/100 g)	Uncertainty**
16:0	10.607	0.003	10.65	0.17
18:0	2.917	0.005	2.87	0.07
18:1	24.461	0.013	24.14	0.28
18:2	57.051	0.017	56.66	0.54
18:3	4.286	0.017	4.68	0.21
20:0	0.368	0.003	(0.3)*	
20:1	0.246	0.003	(0.2)*	

* = Indicative values, not certified

** = Uncertainty is taken as half-width of the 95% confidence interval of the certified mean value.

Table 4. Quantitative Data of Detected Components for CRM 164

Fatty acid	Measured concentration (g/100 g)	Standard deviation	Certified concentration (g/100 g)	Uncertainty*
4:0	3.522	0.012	3.49	0.06
6:0	2.318	0.003	2.36	0.19
8:0	1.420	0.002	1.36	0.10
10:0	3.010	0.006	2.89	0.12
12:0	3.907	0.008	4.03	0.10
14:0	11.383	0.014	10.79	0.35
16:0	27.693	0.005	26.91	0.84
18:0	10.882	0.009	10.51	0.40
18:1	24.832	0.009	24.82	0.61
18:2 (Σ)	2.844	0.012	2.68	0.40
18:3	0.604	0.005	0.51	0.04

* = Uncertainty is taken as half-width of the 95% confidence interval of the certified mean value.

Conclusions

This method, using an Agilent DB-Wax column and Agilent 5975T GCMS is useful for the analysis of classical edible oils and fats in the field when fast and timely response is required, including the determination of butyric acid in milk fat. Using retention time locking, retention times can be stable and provide real information on identification between instruments. RTL database searching makes peak identification more accurate.

For More Information

These data represent typical results. For more information on our products and services, visit our Web site at www.agilent.com/chem.

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