Application Note: 10058

Determination of Fatty Acid Methyl Esters by Ultra Fast GC: 20-fold Reduction in Analysis Time

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Key Words

- Fatty Acid Methyl Esters (FAME)
- Ω-3 Fatty Acids
- 20-fold speed increase
- Ultra Fast GC

Introduction

The determination of Fatty Acids is carried out worldwide to obtain direct information regarding the fat composition of various food matrices, such as seafood, vegetables, and olive oil. The distribution of fatty acids, providing a unique fingerprint for a given food product, is usually controlled as an index of quality in order to protect against adulteration.

This analysis is generally performed by gas chromatography following the conversion of the Fatty Acids into their corresponding Methyl Esters (FAME). The GC separation, using conventional methods, typically requires 30 to 60 minutes.

This note provides a method to determine FAME by using the TRACE™ GC Ultra equipped with the Ultra Fast option (Figure 1). The new technology developed by Thermo Electron Corporation allows the GC to achieve unprecedented heating rates and dramatically reduce the cooling time. A complete analytical cycle can hence take place in a couple of minutes with a 20-fold speed increase over the conventional GC method.

Standard and real samples of FAME extracted from different matrices have been tested to demonstrate the accuracy, repeatability, and precision of the method. All of the analyses have shown excellent accordance with the results obtained by the conventional GC methods.

Ω-3 Fatty Acids

A particular interest concerns the Omega-3 fatty acids, EPA and DHA, which consist of the all cis forms of 5,8,11,14,17-eicosapentaenoic acid and 4,7,10,13,16,19-docosahexaenoic acid, respectively. These acids are well known to effectively help promote a superior cardiovascular system, healthy cholesterol and triglycerides levels, and healthy brain and memory functions. Their application as pharmaceutical compounds for people affected by heart disease is now consolidated in many therapies. Moreover, recent findings from cancer research suggest that a diet rich in these “good fats” (only seafood, particularly salmon, features a large content of Omega-3) may also contribute to the prevention of prostate cancer and breast cancer [1].

Ultra Fast GC Configuration

For this application, the TRACE GC Ultra is configured with a SSL injector, the Ultra Fast option (including the analytical column), and a Fast Flame Ionization Detector (FFID) featuring 6 ms as the detector’s response time to signal and acquisition frequencies up to 300 Hz. Such a high speed is, in fact, a compulsory requirement for the correct acquisition (15-20 points/peak) of the extremely narrow peaks (approx. 100ms $PW_{1/2}h$) typical for this type of chromatography [2].

The column module, connected to the Split-Splitless injector and the FID detector as a removable accessory, is completely and directly controlled by the instrument local user interface and electronics. The same Gas Chromatograph has been used to perform the same application in conventional mode with the easy removal of the Ultra Fast accessory.

All the samples have been injected through the TriPlus™ Autosampler (Figure 2) using the liquid band formation technique [3,4], automatically selectable on the control software. This technique implies the presence of a plug of glass wool in the middle of the liner, in order to retain all the liquid and enable the beginning of the evaporation process from a single droplet.

The wool also plays an important role in preserving the column’s efficiency: the non-evaporating by-products, eventually present, remain trapped on the wool layer without entering the column. The use of glass wool is strongly suggested only for samples that do not contain thermo-labile components, which could undergo degradation catalyzed by the wool itself.

The TriPlus Autosampler, through the “Rapid Mode”, can be programmed to perform a syringe-cleaning phase during the analytical run of the previous sample in the sequence. The outcome is the elimination of the dead time existing between two consecutive runs, further reducing the total run-to-run time and increasing productivity.

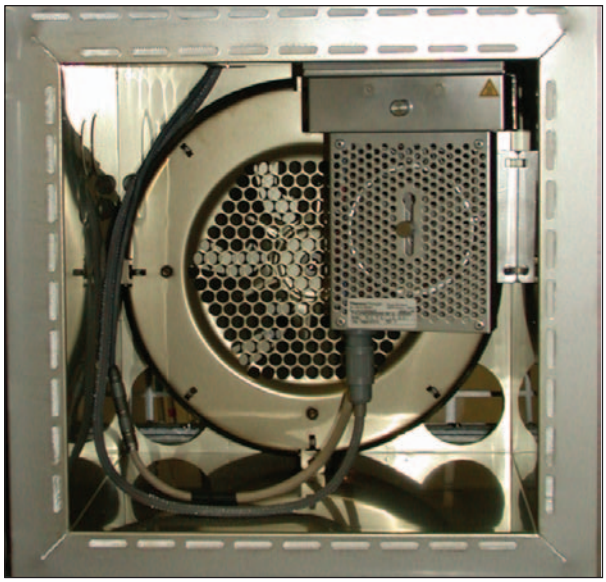

Figure 1: TRACE GC Ultra with the Ultra Fast Module

Figure 2: TriPlus Autosampler installed on a TRACE GC Ultra

Sample Preparation

The analysis method requires the conversion of the triglycerides contained in the raw oil sample into Fatty Acid Methyl Esters through transesterification and their separation by liquid-liquid extraction. Serum analysis involves the extraction of lipids from serum, purification by thin layer chromatography, and finally the conversion to FAME.

Results and Discussion

Standard FAME Analysis

A standard solution of FAME, 50 ng/µL in iso-octane, particularly rich in a critical pair of Omega-3 fatty esters is analyzed through a CarboWax column, 5 m long, 0.1 mm i.d., 0.1 µm film thickness (p/n UFMC00001010501). The column temperature is programmed from 120°C (6 sec) to 250°C (12 sec) @ 1.7°C/sec. The injection is performed with a split ratio 1:50 and constant flow operating mode at 0.8 mL/min (hydrogen used as carrier gas, after providing the GC unit with a hydrogen sensor). The injector temperature and the heated block temperature are both set at 250°C. The injected volume is 1µL.

Figure 3 reports the chromatogram acquired in Ultra Fast mode, compared with that acquired in conventional mode (through a CarboWax, 25 m long, 0.25 mm i.d., 0.2 µm f.t.; temperature program: 90°C (2 min) to 150°C @ 30°C/min, to 225°C (6 min) @ 3°C/min, total time 33 minutes). The 17 peaks elute in 96 seconds instead of 33 minutes, with over 20-fold gain in time, when using the Ultra Fast Module.

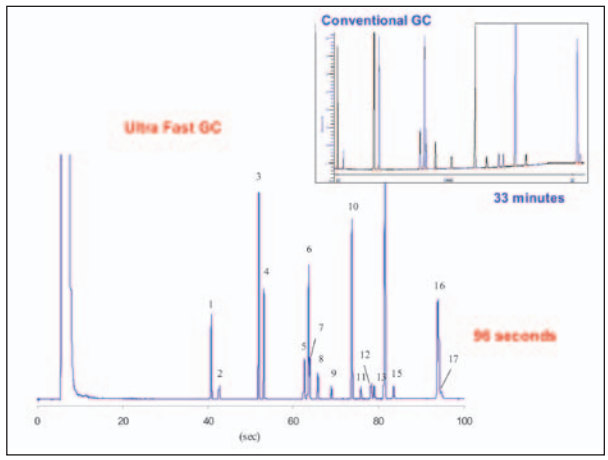

Figure 3: FAME standard mix analyzed with UFM and in conventional mode.

Table 1 presents the quantitative results, pointing out high recoveries towards the theoretical values and showing excellent repeatability of both peak areas and retention times. In the tables, the two most important Omega-3 fatty esters are highlighted in red bold.

	COMPONENT	AVERAGE %	THEORETICAL RESPONSE	RECOVERY	AREA RSD%	RETENTION TIME (SEC)	RT STANDARD DEVIATION (SEC)
1	C 14: 0	5.78	5.64	1.025	1.3	40.72	0.09
2	C 14:1n-5	0.94	0.95	0.988	2. 5	42.65	0.08
3	C 16:0	14.78	14.47	1.021	1.6	51.93	0.09
4	C 16:l n-7	7.74	7.77	0.996	1.7	53 .18	0.09
5	C18:0	2.99	2.95	1.013	1.5	62.59	0.08
6	C 18:1n-9	10.29	9.90	1.039	1.9	63.53	0.08
7	C 18:1n-7	2.72	2.97	0.916	2.8	63.87	0.08
8	C18:2n-6	1.96	1.99	0.986	2.8	65.75	0.08
9	C18:3n-3	0.99	1.00	0.989	2.7	68.95	0.07
10	C20:1n-9	14.23	14.07	1.011	1.8	73.77	0.06
11	C20:2n-6	0.97	1.01	0.958	3.2	75.94	0.06
12	C20:4n-6	1.19	1.02	1.168	4.0	78.28	0.06
13	C20:3n-3	1.00	1.02	0.979	2.4	79.07	0.06
14	**C20:5n-3**	19.14	19.57	0.978	1.7	81.50	0.06
15	C22:1n-9	1,00	1.02	0,981	5.3	83.54	0.05
16	**C22:6n-6**	13.24	13.61	0.973	2.3	93.98	0.04
17	C24:1	1.05	1.03	1.015	6.0	94.70	0.05

Table 1: FAME standard mix; comparison of UFM results with theoretical values (sequence of 5 runs).

Fish Oil Analysis

Fish oil analysis is performed with the same column module and analytical conditions used for the FAME standard analysis described in the previous paragraph. Figure 4 reports the related chromatogram with identification of 12 components.

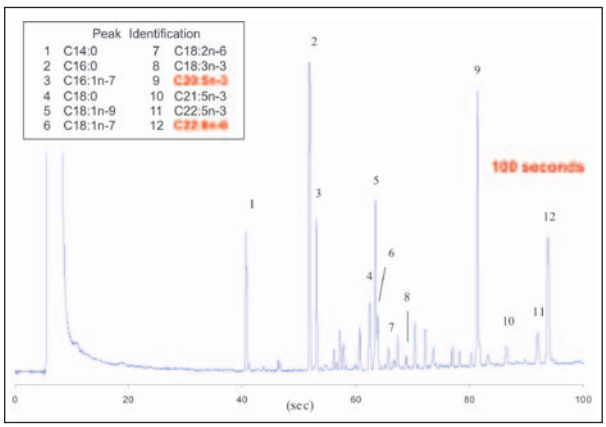

Figure 4: Ultra Fast GC chromatogram of a fish oil sample with peak identification table.

To test the robustness of the system, about 100 analyses of real fish oil samples were performed, and the sequence was completed with five analyses of FAME standard solution injected under the same analytical parameters. Top levels of repeatability and accuracy, together with the continuation of optimal peak shapes obtained on these last 5 standards, prove that column efficiency is preserved even after numerous injections of real matrix samples. This is due to the presence of a layer of glass wool in the middle of the liner where the non-evaporating by-products remain trapped without entering the column.

Olive Oil Analysis

This analysis is carried out through a CarboWax column, 5m long, 0.1 mm i.d., 0.2 µm film thickness (p/n UFMC00001010503); this column features a higher capacity than the one used for the fish oil samples. One µL of Olive Oil FAME extract is analyzed in Ultra Fast mode, with a temperature program ranging from 150°C (10 sec) to 250°C (20 sec) @ 1.7 °C/sec. The injection is performed with a split ratio 1:300 and constant flow operating mode at 1.0 mL/min (helium used as carrier gas). The injector temperature and the heated block temperature are both set at 250°C.

The analysis of the same sample is repeated in conventional mode with a column featuring the same stationary phase, but a different geometry: 25 m long, 0.25 mm i.d., 0.2 µm film thickness. The same temperature range is covered at 5°C/min.

Figure 5 reports the chromatograms from each analysis, and Table 2 the perfect accordance between the two analytical results: the same accuracy is achieved in less than 90 seconds in Ultra Fast mode, instead of 20 minutes using conventional gaschromatography.

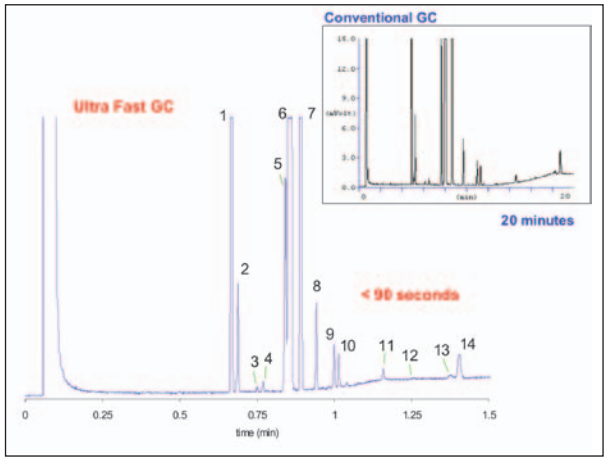

Figure 5: Olive Oil sample analyzed with UFM and in conventional mode.

COMPONENT		AREA % UFM	AREA % CONVENTIONAL GC
1	C16:0	12.58	12.61
2	C16:1	0.82	0.79
3	C17:0	0.04	0.05
4	C17:1	0.09	0.08
5	C18:0	2.33	2.38
6	C18:1n-9c	71.38	71.58
7	C 18:2n-6c	10.02	10.29
8	C18:3n-6	0.78	0.77
9	C20:0	0.42	0.42
10	C20:1	0.32	0.33
11	C22:0	0.15	0.14
12	C23:0	0.04	0.03
13	C24:0	0.09	0.06
14	C24:1	0.56	0.55

Table 2: Olive Oil sample; comparison of UFM results with those obtained in conventional GC mode.

Serum Analysis

There is a correlation between dietary fat intake and serum fatty acids composition [5]. Determination of fatty acid composition in serum phospholipids is considered a good marker of medium-term dietary fat intake (within weeks), which can be used to evaluate the compliance to dietary intervention studies and the effect of diet. The GC analytical determination constitutes a very time-consuming part of the studies. In this view, an Ultra Fast GC method is an important tool since it allows for a significant reduction in analysis time.

Serum analysis is carried out using the 5 m, 0.1 mm i.d., 0.2 µm film thickness UFM-CarboWax column module (p/n UFMC00001010503). Two µL of the serum extract are injected in split mode with a split ratio of 1:250. Temperature program is: 160°C (10 s) to 200°C @ 1.3°C/s, to 230°C @ 0.7°C/s and finally to 250°C (60 s) @ 0.6°C/s. Hydrogen at 0.2 mL/min is used as carrier gas.

Figure 6 reports the example of a chromatogram of serum extract with the identification of 12 FAME.

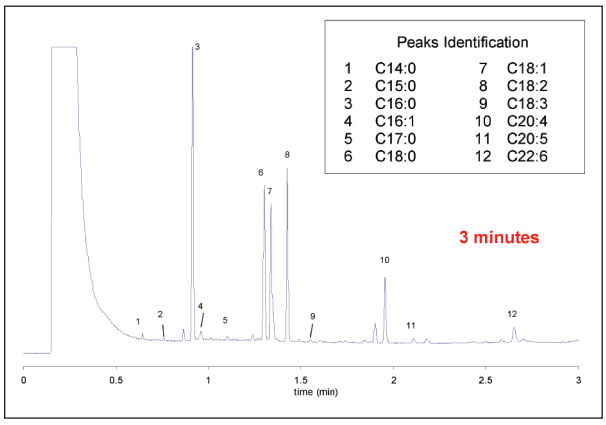

Figure 6: Ultra Fast GC chromatogram of a serum extract with peaks identification table.

Conclusions

The TRACE GC Ultra equipped with Ultra Fast option allows the operator to achieve the determination of Fatty Acid Methyl Esters with up to a 20-fold speed increase over conventional GC methods with excellent accuracy and precision of both peak areas and retention times. This Ultra Fast system, tested with different sample matrices, has clearly proved to be a robust and reliable alternative to conventional GC, as seen in the repeatability of accurate results.

References

1. H. Bartsh, et al., Carcinogenesis, 20 (1999) 2209.

2. P. Magni, R. Facchetti , D. Cavagnino and S. Trestianu, Proceedings of 25th International Symposium of Capillary Chromatography, KNL05, Riva del Garda, Italy, May 13-17, 2002, ed. P. Sandra.

3. F. Munari, S. Trestianu in Proc. 4th Int. Symp.Capillary Chromatography, Hindelang Germany, R. E. Kaiser (ed), Hüthig, Heidelberg, 1981, p. 349.

4. K. Grob, M. Biedermann, J.Chromatogr. A, 2000, 897, 237-246.

5. P. Avons, Markers of the Dietary Supply of Fatty Acids. Their Use in Epidemiologic Surveys.

Rev Epidemiol Sante Publique, 1985; 33 (4-5): 312-23.

Acknowledgements

The application has been developed through the effective collaboration with:

A. Cavalleri and C. Colombo, Istituto Nazionale per la Cura e lo Studio dei Tumori, located in Milan, Italy;

Terje Aasoldsen, SAMSI Analytical Application Services, based in Norway;

who have provided all of their expertise in this application field.

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