AppNote 3/2013

Automated Determination of Total Fat, Saturated Fat, Monounsaturated Fat and Trans Fat Content in Food Samples

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Keywords
Automation, total fat, trans fat, saturated fat, foods, gas chromatography, flame ionization detection, microwave, saponification, FAME, FAMEs.

Abstract
Determination of total fat, saturated fat, monounsaturated fat and trans fat content in food samples is necessary for complying with applicable food labeling requirements. A typical procedure for saponification of the sample involves refluxing with sodium methoxide in methanol, followed by a second reflux with boron trifluoride in order to esterify the free fatty acids. Prior to injection into the gas chromatograph, the fatty acid methyl esters (FAMEs) must be extracted from the reaction mix and the extract must be dried. The reflux times are typically an hour. After the sample is prepared, the round bottomed flask and condenser must be cleaned. This process is laborious and time consuming, which limits sample throughput.

A combined autosampler and liquid handling robot, which is commonly used for a wide variety of sample preparation techniques can be interfaced directly to a CEM Discover SP-D microwave unit. In this way, a single integrated system under MAESTRO software control can perform saponification of fats in combination with further sample preparation steps and finally introduction to a GC or HPLC system.

Introduction

In this work, we demonstrate an automated saponification/esterification sample preparation using a GERSTEL MultiPurpose Sampler (MPS) coupled to a CEM Discover SP-D microwave. The use of the microwave, in place of refluxing, allows a significant reduction in the time required for the saponification/esterification reactions, such that the entire sample preparation procedure can take place within the timeframe of the gas chromatographic run. This enables efficient overlap of the sample preparation and sample analysis times for maximum throughput. The MPS is coupled directly to a GC-FID to streamline the entire extraction and analysis process, as well as to eliminate laboratory personnel exposure to potentially hazardous materials. Several food types were analyzed to demonstrate the usefulness of this process.

Experimental

Instrumentation. Analyses were performed on an Agilent 7890 equipped with a Flame Ionization Detector (FID), PTV inlet (CIS 4, GERSTEL), Discover SP-D Microwave (CEM) and a single rail, dual head MultiPurpose Sampler (MPS, GERSTEL).

Analysis conditions.

PTV:	baffled liner
	split (50 mL/min)
	40°C; 12°C/s; 260°C (3 min)
Column:	100 m CP Sil-88 (Agilent)
	d_i = 0.25 mm d_f = 0.20 µm
Pneumatics:	He, constant flow = 1.2 mL/min
Oven:	80°C (2 min); 4°C/min;
	225°C (25 min)
FID:	260°C

Standard preparation. A 37 component FAME standard (Supelco Catalog Number 47885-U) was used for this study. An aliquot of the FAME standard was put into a low volume insert in a 2 mL autosampler vial. A tritridecanoin (NU-CHEK, Inc, Catalog Number T-135) was prepared by weighing 120 mg of standard into a 25 mL volumetric flask and filling it to the mark with chloroform.

Sample preparation. Peanuts, caramel, cheddar cheese, packaged cheese, yogurt, vegetable cheese spread, and chocolate samples were used for this study. The peanut sample was placed in a coffee grinder for approximately 30 seconds. Between 0.1-0.3 grams of sample was weighed into a 35 mL microwave vessel. A stir bar was added to the vessel, which was then sealed with a cap.

Automated sample preparation

1. Add 1.0 mL of internal standard solution (Tritridecanoin in $CHCl_3$)
2. Add 4 mL of methanolic base, 0.5N
3. Microwave for 5 minutes at 80°C
4. Add 5 mL of BF_3 in methanol
5. Microwave for 5 minutes at 80°C
6. Add 5 mL of Hexane and 10 mL of water
7. Stir for 3 minutes
8. Transfer 1 mL of the hexane layer to a 2 mL vial filled with 0.20 g of Na_2SO_4
9. Mix for 1 minute at 500 rpm
10. Inject 1.0 µL into the gas chromatograph

Sample introduction. The samples were placed in a VT15-40, 15 position tray on the MPS. For each analysis, a one microliter aliquot of the processed sample was introduced into the GC using a cold split (50:1) injection.

Results and Discussion

Figure 1 shows a picture of the system used for this study. A 5.0 mL syringe was used in the left autosampler tower (injection unit) and a 10 µL syringe in the right tower. Figure 2 shows a chromatogram for the 37 component FAME standard with peaks identified. Peaks were identified by absolute retention time and retention time relative to the internal standard. Figures 3-5 show example chromatograms for the yogurt, cheddar cheese, and vegetable cheese spread samples, respectively.

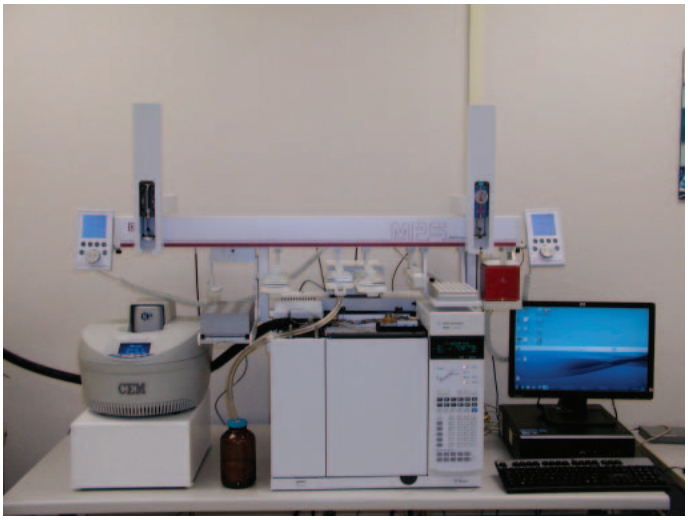

Figure 1. GERSTEL MultiPurpose Sampler (MPS) with CEM Discover SP-d Microwave

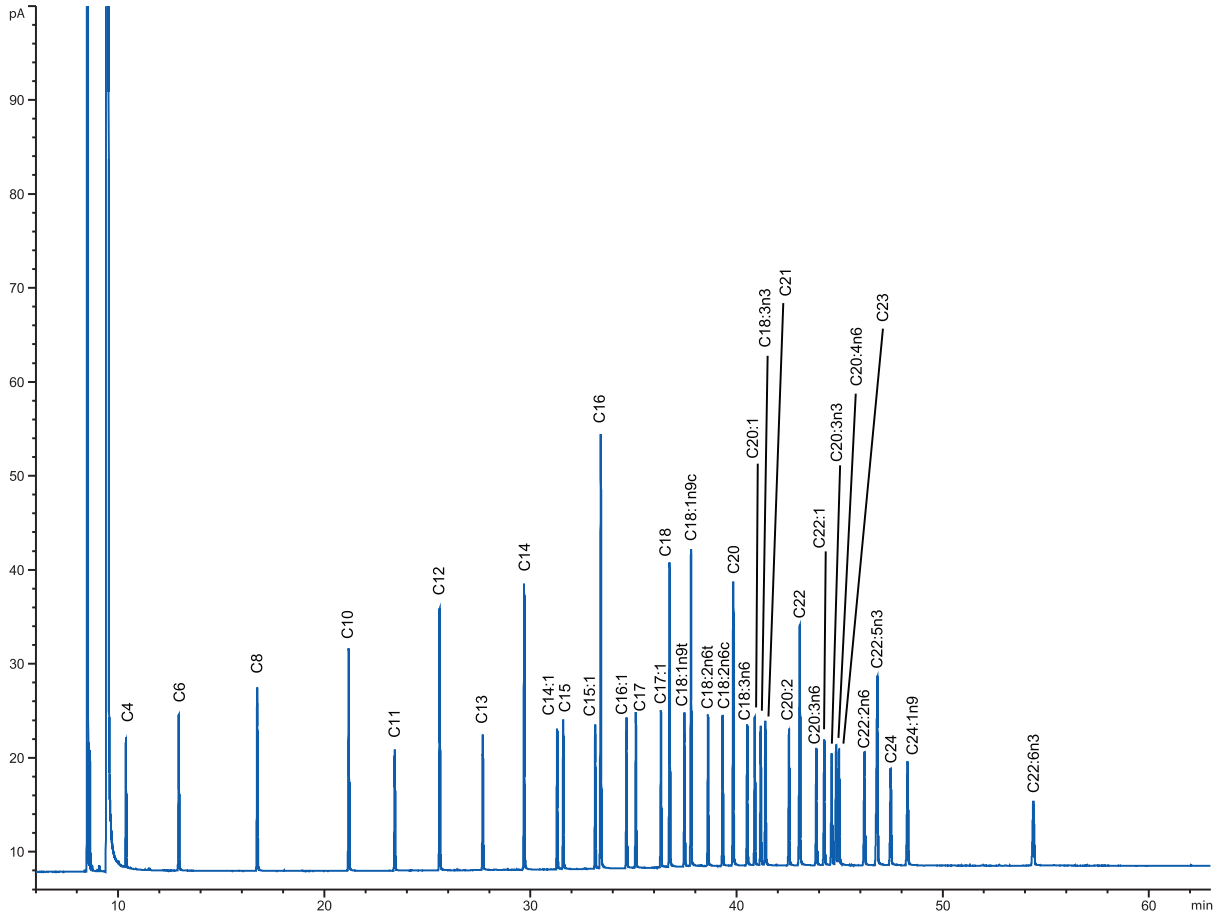

Figure 2. Chromatogram of the 37 component FAME standard.

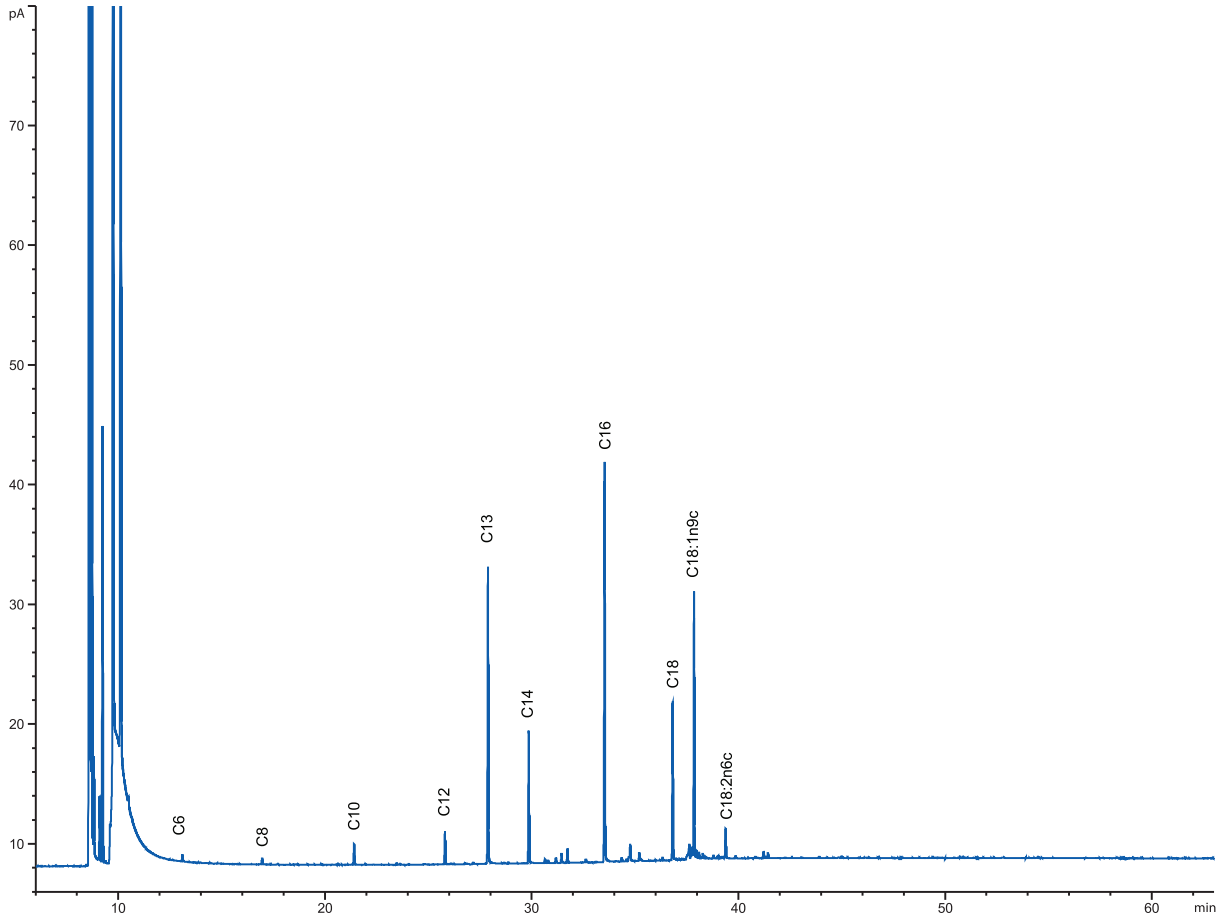

Figure 3. Chromatogram of a yogurt sample.

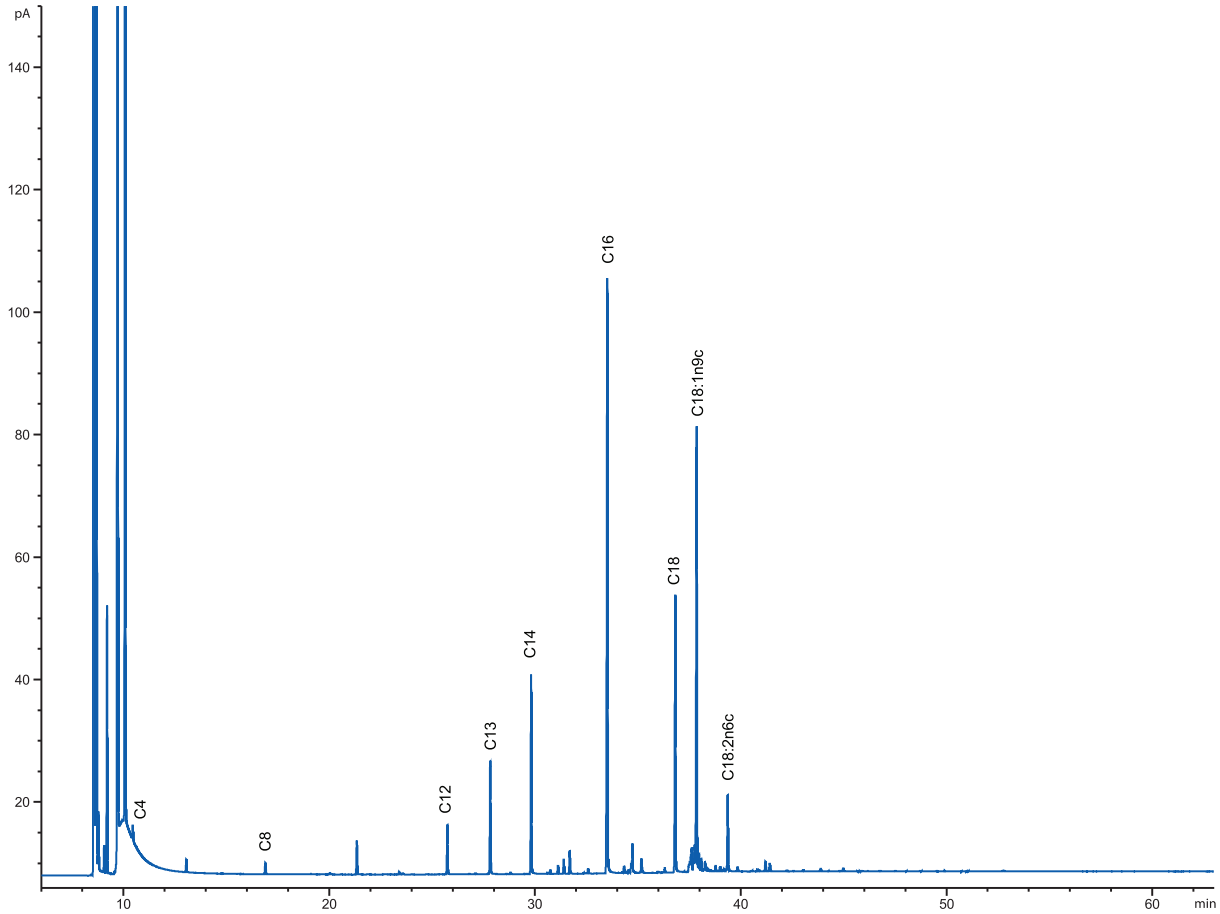

Figure 4. Chromatogram of a cheddar cheese sample.

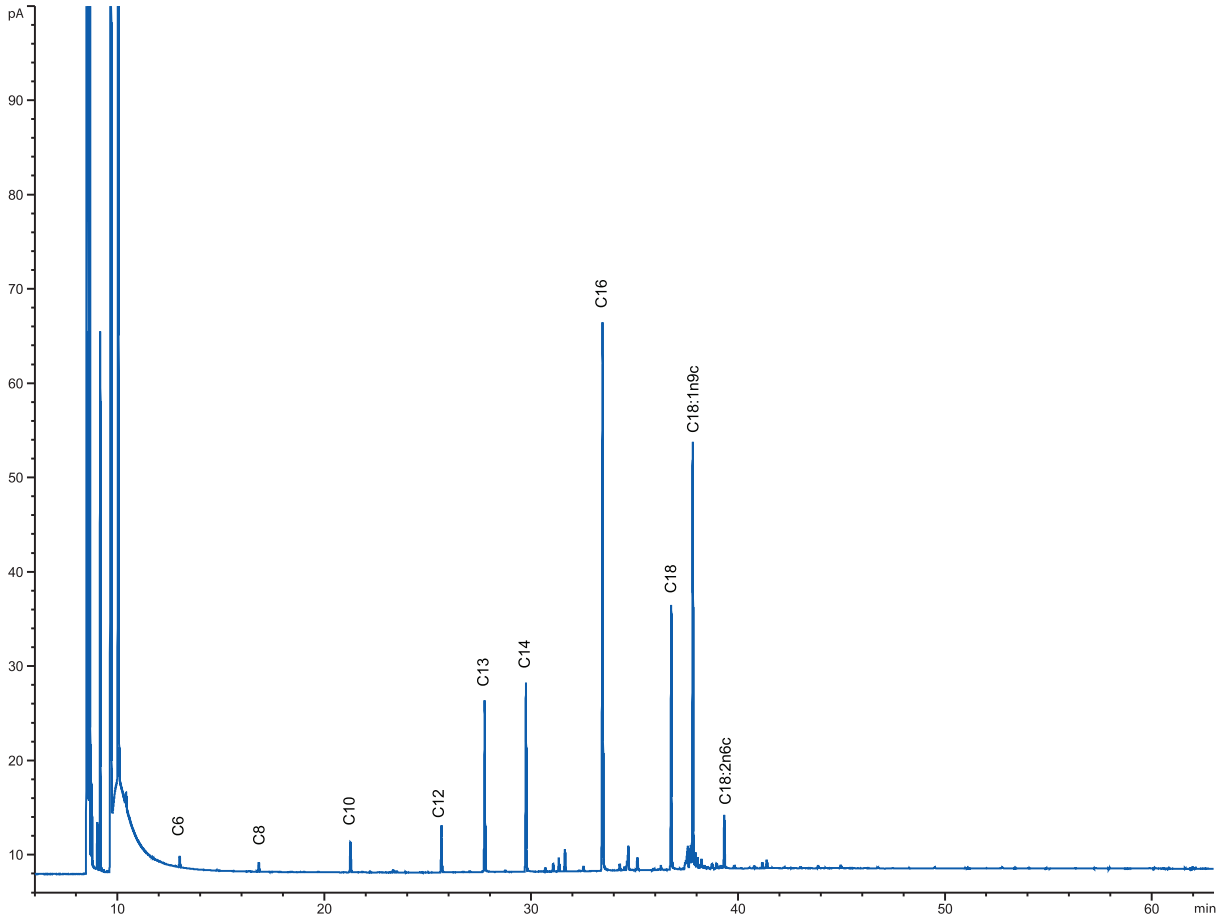

Figure 5. Chromatogram of vegetable cheese spread sample.

The peanut, caramel, and chocolate samples were run in triplicate. The yogurt, cheeses, and cheese spread were run in duplicate. The chromatograms were integrated and the peak area data exported to an Excel worksheet. The relative response factors, R_f, for the FAMEs were calculated from the 37 component FAME standard using the equation:

$$R_f = \frac{A_i}{A_{std}} \times \frac{W_{std}}{W_i}$$

where A_i is the peak area of an individual FAME, A_{std} is the peak area of the C13 peak, W_{std} is the weight of C13 in the FAME standard and W_i is the weight of the FAME in the standard. The FAME standard was injected at the beginning and end of the sequence. The average of the two values was used for the R_f calculation.

The weight of individual FAMEs in the sample is calculated from:

$$W_{FAME} = \frac{A_i \; x \; W_{std} \; x \; 1.0059}{A_{std} \; x \; R_f}$$

where A_i is the peak area of an individual FAME, A_{std} is the peak area of the C13 peak, W_{std} is the weight of C13 added to the sample and R_f is the relative response factor. The number 1.0059 is the conversion factor for Triglyceride to FAME conversion for C13.

The W_{FAME} is converted to weight of triglyceride by multiplying by a conversion factor. The conversion factors were taken from Reference [1]. The total percent fat, as triglycerides, was calculated by summing all the individual triglyceride weights, multiplying by 100, and dividing by the sample weight.

The weight of each fatty acid is calculated by multiplying W_{FAME} by an appropriate conversion factor. The conversion factors were taken from Reference [1]. The total percent saturated fat was calculated by summing all the individual free fatty acid weights for the saturated FAMEs detected, multiplying by 100, and dividing by the sample weight.

The total percent fat, as acids, was calculated by summing all the individual acid weights, multiplying by 100, and dividing by the sample weight.

The total percent monounsaturated fat was calculated by summing all the individual free fatty acid weights for the monounsaturated FAMEs detected, multiplying by 100, and dividing by the sample weight.

The total percent polyunsaturated fat was calculated by summing all the individual free fatty acid weights for the polyunsaturated FAMEs detected, multiplying by 100, and dividing by the sample weight.

The total percent trans saturated fat was calculated by summing all the individual free fatty acid weights for the trans saturated FAMEs detected, multiplying by 100, and dividing by the sample weight.

The results for the sample are given in Table 1. The results show good precision for the three samples run in triplicate. The standard results are also shown in Table 1. The results for the automated microwave method compare well with the values obtained using a standard method.

Table 1. Standard and experimental sample fat values.

Experimental Values	Chocolate Average (% RSD)	Peanut Average (% RSD)	Caramel Average (% RSD)	Cheddar Cheese	Packaged Cheese	Yoghurt	Vegetable Cheese Spread
Total Fat, as Triglycerides	26.5 (2.0)	47.3 (1.3)	7.17 (4.0)	33.1	13.1	7.31	24.8
Total Fat, as Acids	25.4 (2.0)	45.3 (1.3)	6.86 (4.0)	ND	ND	ND	ND
Saturated Fat	17.1 (1.9)	8.87 (2.6)	5.20 (5.6)	20.7	8.15	4.71	15.4
Monounsaturated Fat	7.99 (2.2)	35.9 (1.4)	1.43 (4.3)	8.27	3.39	1.80	6.42
Polyunsaturated Fat	0.88 (2.6)	4.62 (3.2)	0.14 (3.5)	1.27	0.39	0.21	0.76
Trans Fat	0.18 (1.8)	0.58 (7.3)	0.22 (4.5)	1.29	0.51	0.26	1.00
Standard Values	**Chocolate**	**Peanut**	**Caramel**	**Cheddar Cheese**	**Packaged Cheese**	**Yoghurt**	**Vegetable Cheese Spread**
Total Fat, as Triglycerides	25.9	49.7	6.1	30.6	12.8	6.70	28.9
Total Fat, as Acids	24.7	47.6	5.8	ND	ND	ND	ND
Saturated Fat	16.0	6.7	4.1	17.7	7.44	3.87	15.5
Monounsaturated Fat	7.7	37.1	1.5	6.12	2.62	1.26	5.49
Polyunsaturated Fat	1.0	3.8	0.2	1.11	0.37	0.16	0.77
Trans Fat	0.1	0.2	<0.1	1.39	0.55	0.25	1.25

The C18 FAME region can be very complicated due to the large number of isomers. For this study, the region before C18:1n9c was integrated as the trans peak, and the region from C18:1n9c to C18:2n6t integrated as the cis peak. This is shown in Figure 6.

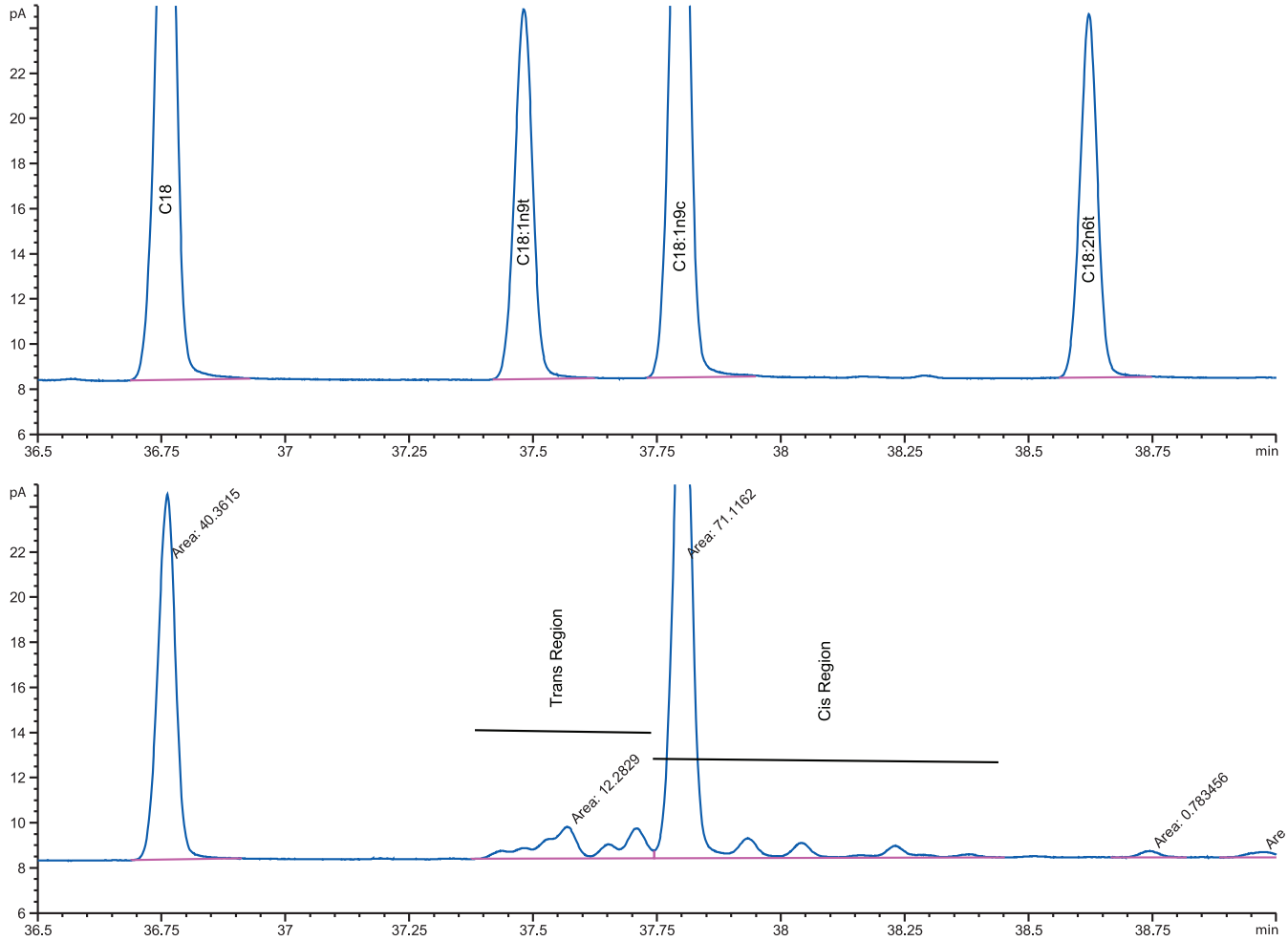

Figure 6. Enhanced view of C18 FAME region.

The GC runtime is 63 minutes long which is sufficient time for preparation of the next sample. The GERSTEL Sequence Scheduler is shown below for 15 samples. The approximate run time for this number of samples along with the standard is 18 hours and 13 minutes.

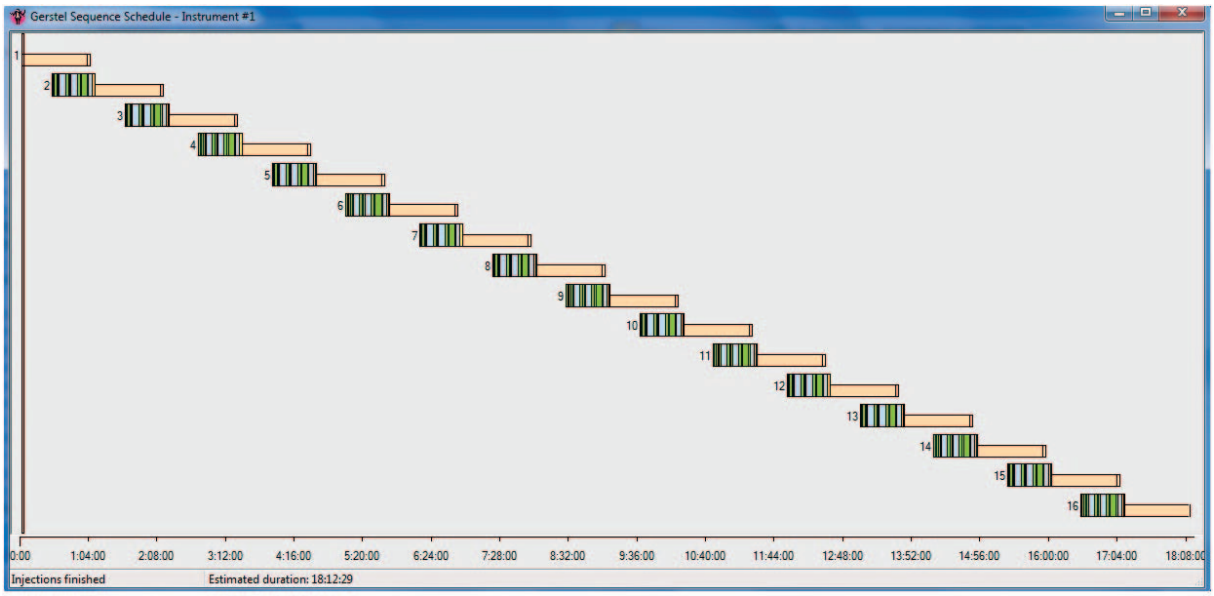

Figure 7. GERSTEL Sequence Scheduler, here shown for 15 samples.

Conclusions

The GERSTEL MPS with integrated CEM Discover SP-D microwave provides an automated sample preparation alternative for the determination of fat content in various food products.

References.

[1] AOAC Official Method 996.06 “Fat (Total, Saturated, and Unsaturated) in Foods”.

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