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Evaluation of fast gas chromatography and gas chromatography–mass spectrometry in the analysis of lipids

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Abstract

Fast and conventional gas chromatography (GC) techniques were applied to nine different lipidic matrices (butter, lard, tallow, and peanut, corn, sunflower, soya, olive, menhaden oils). Simultaneous methylic transesterification was performed on all samples prior to GC analysis. Several practical aspects concerning high speed analysis were investigated, such as the great increase in linear velocity, the use of fast temperature ramps, column sample capacity and detection systems. Analytical results showed certain losses in resolution, balanced by a consistent reduction in analysis time. The actual time savings were variable (60–70 min) as they were dependent on the complexity of the sample while the speed enhancement factor was equal to 10.5. Peak identification was achieved by means of different information sources, such as fast GC–mass spectrometry (MS), linear retention indices and comprehensive two-dimensional (2D) gas chromatography group patterns. The method developed was shown to be applicable in routine applications on complex natural samples.

Keywords: Gas chromatography, fast; Gas chromatography, comprehensive two-dimensional; Lipids; Fatty acid methyl esters

1. Introduction

Lipids are used on a daily basis in our diet, consumed either as foods constituents or as condiments, and they have a fundamental importance in keeping us in good health. From a physiological standpoint, they play a role in several biochemical functions, such as constituents of biological membranes, vitamins, hormones, bile acids and as energy suppliers.

In the last several years, the correlation between lipidic intake and health has been highlighted by several works regarding the links between cardiovascular diseases and a diet rich in saturated fatty acids (SFAs) [1–3]. This has led some nutritionists to emphasize the potential benefits of the Mediterranean diet, rich in vegetables, sea products and extravirgin olive oil. All these food products, in fact, contain a consistent amount of monounsaturated fatty acids (MUFAs) and essential fatty acids (EFAs), the so-called "good lipids", due to their capability of hindering the formation of atheromatose plaques. On the other hand, the assumption of a diet rich in polyunsaturated fatty acids (PUFAs) and SFAs is not recommended, as the former tend to oxidize while the latter to settle in blood vessel walls. For the aforementioned reasons, there is a need to increase the knowledge about lipids in food, and analytical chemistry can be a precious support to both medicine and food science.

The determination of the fatty acid composition in fats and oils, present mainly as triacylglycerols, is generally achieved by open tubular column (OTC) gas chromatography via methylic transesterification of the lipidic matrix [4]. This well-established conventional GC method produces effective results but has one substantial limitation: the cost in time. In fact, satisfactory separations concerning moderately complex matrices such as lipids, can take an hour or more. This factor becomes particularly important for those laboratories where a great number of analyses are carried out and/or where there is a need for quick results. The growing necessity, over the years, for fast GC techniques has seen the

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application of several different theories with the general aim of decreasing analysis time while contemporaneously maintaining acceptable efficiency for a given separation [5–10]. The most successful instrumental tool in this direction has been the micro-bore capillary column [11,12]. Although the theory supporting a reduction in column internal diameters as a route towards high speed analysis was formulated more than 40 years ago [13], their routine use in fast GC is quite recent. In comparison to traditional columns, they are characterized by a higher resolving power and thus are capable of maintaining the same efficiency as these while working at a higher speed. The increase in solute velocity is obtained through the application of high inlet pressures and fast temperature program rates. These instrumental requirements have seriously delayed the use of fast techniques because of the lack of adequate equipment. The definitions used in rapid GC analysis (fast, very fast, ultra fast) have been defined in literature by different authors and are based on parameters such as analysis time, column efficiency, speed enhancement factor (SEF) and peak half width measures [14–18]. The term "fast" was used to define the rapid GC technique applied in this research.

The present investigation had various aims, the first being an evaluation of the loss in resolution as linear velocities and temperature rates were raised. This, in order to define ranges in which speed and an acceptable separating power are compatible. Furthermore, the most suitable flame ionization detection (FID) sampling frequency was individuated in order to obtain correct integration for all fast analyses peaks. One of the main fast GC characteristics is, in fact, the minimization of band broadening which means that narrow solute bands pass very rapidly through the detection system. The consequential formation of high and narrow peaks leads to a higher signal-to-noise ratio and to an improved detectability of analytes in comparison to traditional methods (if sample amounts are compared) which, on the other hand, produce broader peaks. Another aspect measured was the reproducibility of chromatographic fast data. In this case, the doubts concerned possible column overloading caused by analyses run in sequence and/or alterations due to the alternate rapid heating and cooling of the system. Also to be assessed was the fast GC–mass spectrometry coupling and the capacity of the quadrupole mass spectrometer to supply a sufficient number of spectra per peak for compound identification. An ultimate issue faced was the variation of height equivalent to one theoretical plate (HETP) and *N* values, respectively, in function of different linear velocities and sample quantities. An accurate evaluation of these factors in fast GC is particularly important as it forms the basis for method optimization. In this work, various lipidic matrices that ranged from relatively simple substances such as olive and corn oil to the rather more complex menhaden oil and butter were analyzed. The samples were prepared simultaneously as high time costs in this analytical step would diminish the importance of high speed GC analysis.

2. Experimental

2.1. Standard

$C_{8:0}$–$C_{36:0}$ hydrocarbons in *n*-hexane solutions (0.1 μg/ml) were purchased from Supelco (Milan, Italy).

2.2. Samples

All the fats and oils analyzed (butter, lard, tallow, and peanut, corn, sunflower, soya, olive, menhaden oils) were commercially available.

2.3. Sample preparation

The samples underwent a simultaneous transesterification in order to obtain the FAMEs: the fats (the oils were not subjected to this step) were brought to a temperature of 40 °C and dehydrated by filtering with Na_2SO_4. One milliliter of the oil was added to 1 ml of a 10% (v/v) solution of concentrated H_2SO_4 in MeOH, then heated at 110 °C for 2 h in closed vials. After cooling, the FAME phase relative to each sample was separated from the acidic solution.

2.4. GC analysis

GC analyses were carried out on a Shimadzu GC-2010 gas chromatograph operated with a split/splitless injector and a Shimadzu autosampler AOC-20s and autoinjector AOC-20i (Shimadzu, Milan, Italy). Column: Supelcowax-10, 30 m × 0.25 mm I.D., 0.25 μm film thickness. Temperature program: 50–280 °C at 3 °C/min. Injection temperature: 250 °C. Injection volume: 1.0 μl. Inlet pressure: 100 kPa. Carrier gas: He, linear velocity u: 30.1 cm/s. Column flow: 1.26 ml/min. Split ratio: 1:100. Detection: FID, at 300 °C. H_2 flow: 50.0 ml/min; air flow: 400.0 ml/min; makeup (N_2/air): 50.0 ml/min. Sampling rate: 12.5 Hz. Data were acquired by a GC solution software (Shimadzu).

The same apparatus was used for fast GC analyses, with the exception of the column: Supelcowax-10, 10 m × 0.10 mm I.D., 0.10 μm film thickness. Temperature program: 50–150 °C at 80 °C/min, to 250 °C at 70 °C/min, to 280 °C at 50 °C/min held for 1 min. Inlet pressure: 414.5 kPa. Injection temperature: 240 °C. Carrier gas: H_2, u: 119.9 cm/s. Column flow: 1.82 ml/min. Injection volume: 1.0 μl. Split ratio: 1:200. Detector temperature: 290 °C. Sampling rate: 50 Hz.

2.5. Comprehensive two-dimensional gas chromatography (GC × GC) analysis

GC × GC analyses were performed using a Shimadzu 2010 model gas chromatograph (Shimadzu) equipped with a Shimadzu autosampler AOC-20s and autoinjector AOC-20i (Shimadzu, Milan, Italy), and GC Solution software for data acquisition. The GC was equipped with a

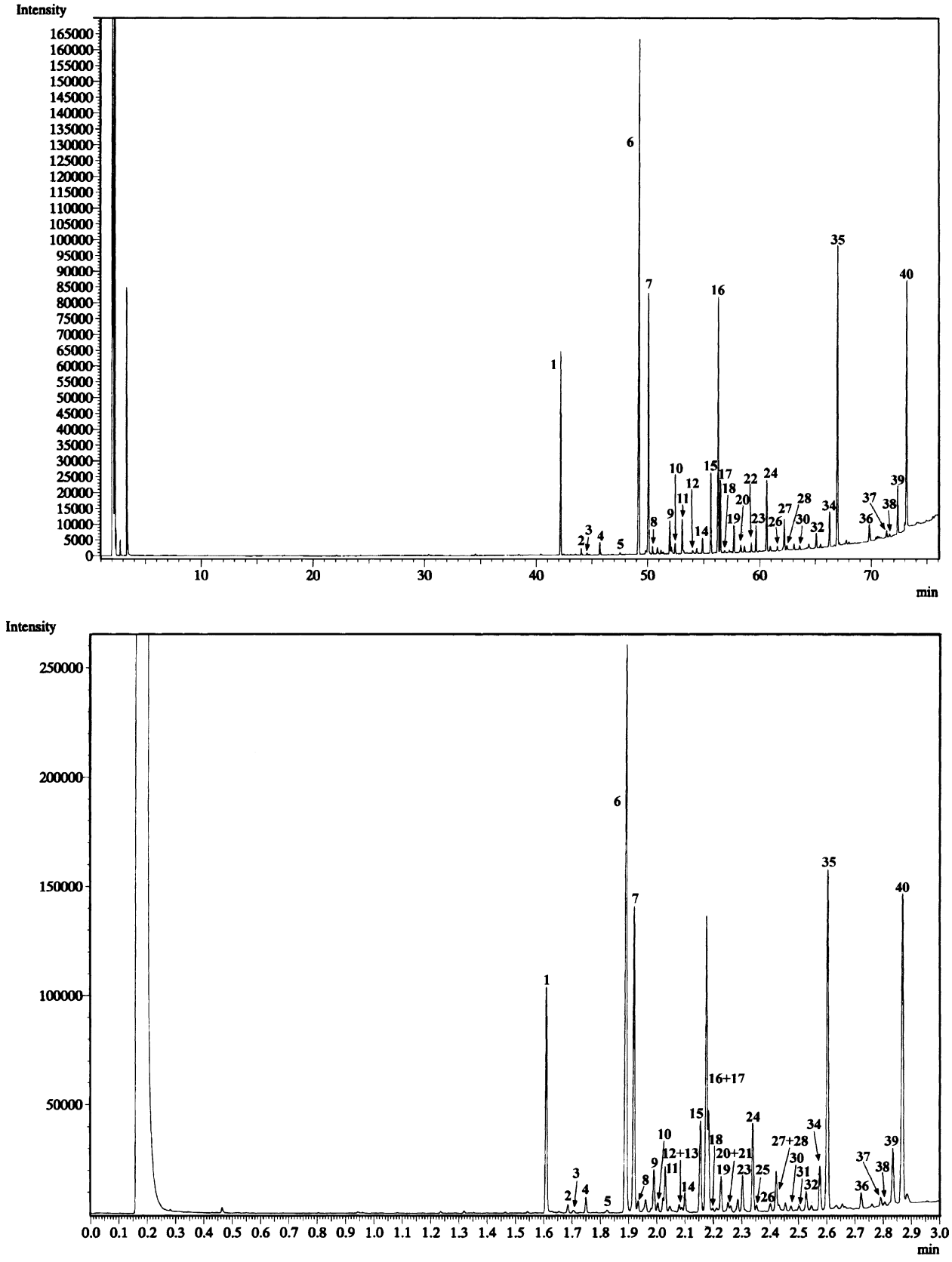

Fig. 1. Conventional and fast GC–FID chromatograms of menhaden oil.

LMCS Everest longitudinally modulated cryogenic system (LMCS; Chromatography Concepts, Doncaster, Australia), with a mechanical stepper motor drive for movement of the cryotrap. A modulation frequency of 0.1666 Hz (6 s cycle) was applied in all analyses and initiated by the GC solution programmed external events that via the electronic controller starts also the motor operation. CO_2 is supplied to the trap, and its expansion cools the trap that is thermostically regulated at about 0 °C. A small internal flow of nitrogen gas (about 10 ml/min) prevents ice formation inside the trap. Data are collected by the GC Solution software and by using its export function. The ASCI data were converted into a matrix with rows corresponding to a 6 s duration, and data columns covering all successive second dimension 6 s chromatograms using the two-dimensional GC Converter 2.0 (Chromatography Concepts). Contour representation of the two-dimensional chromatograms was through Transform version 3.3 software (Fortner Software, VA, USA). The columns set for GC × GC analysis consisted of two columns, which were serially connected by a zero-dead-volume glass press-fit (Mega, Legnano, Italy). In this study, the following column set was used: the conventional first dimension was a Supelcowax-10 (polyethylene glycol) column 30 m × 0.25 mm I.D., 0.25 μm film thickness and the secondary fast column was a SPB-5 (5% diphenyl + 95% dimethyl polysiloxane) 1 m × 0.10 mm I.D., 0.10 μm film thickness (Supelco Italy, Milan, Italy). The operational conditions were as follows: temperature programmed conditions from 200 to 250 °C at 2 °C/min. The GC was equipped with a split/splitless injector (260 °C); an injection volume of 1.0 μl was employed and a split ratio of 100:1 was used. The carrier gas was hydrogen, and the column head pressure was 200 kPa at constant pressure. u: 98.8 cm/s. FID (280 °C). Sampling rate: 50 Hz. H_2: 50 ml/min; air: 400 ml/min; make-up: 50 ml/min (N_2/air).

2.6. GC–MS analysis

For conventional GC–MS analyses, a Shimadzu GCMS-QP2010 (Shimadzu, Milan, Italy) instrument was used, equipped with a 30 m × 0.25 mm I.D., 0.25 μm film thickness RTX-WAX column (Restek, Bellefonte, PA, USA). Temperature program: 50–280 °C at 3 °C/min. Carrier gas: He, at constant pressure: 24.9 kPa, u: 30 cm/s. Column flow: 0.7 ml/min. Injection volume: 0.5 μl. Split ratio: 1:100, injection temperature: 250 °C. Interface temperature: 230 °C. Ionization energy: 1.50 kV. Acquisition mass range: 40–400 *m/z*; solvent cut: 3 min.

Fast GC–MS analyses were carried out on the same instrumention but with the following column: Supelcowax 10 m × 0.10 mm I.D., 0.10 μm film thickness (Supelco Italy). Temperature program: 50–150 °C at 80 °C/min, to 280 °C at 70 °C/min held for 1 min. Carrier gas: He, at constant pressure: 850 kPa, u: 107.4 cm/s. Column flow: 2.9 ml/min. Injection volume: 0.5 μl. Split ratio: 1:200. Injection temperature: 250 °C. Interface temperature: 230 °C. Ionization energy: 1.50 kV. Acquisition mass range: 50–350 *m/z*; solvent cut: 36 s.

3. Results and discussion

Nine FAMEs samples obtained from various fats and oils were each analyzed three times consecutively with both conventional and fast techniques. The significant presence in some of the samples (butter, tallow and lard) of low molecular weight FAMEs imposed the choice of an initial general column temperature of 50 °C. The respective GC run times were 76 min and 180 s. Fig. 1 shows two chromatograms that belong to a conventional and fast analysis carried out on menhaden oil (the most complex of the analyzed matrices). Forty peaks were separated and identified through the application of the conventional method (Table 1). Peak 9 regards a triple peak coelution concerning $C_{16:2\omega4}$, $C_{17:0}$ anteiso and iso, whose attribution was achieved through the exploitation of the high resolving power of GC × GC and its predictive potential [19]. The fast analysis separated 40 compounds of which three pairs and a triplet were partially resolved. Retention times were diminished by an average factor of 25 (Table 1). The R.S.D. values are also reported and they demonstrate the excellent reproducibility of the fast GC application in comparison to that of conventional GC analysis. The maximum R.S.D. values, although higher for the fast GC analysis (0.155% vs. 0.019%), all demonstrated only slight variations between consecutive applications.

As it can be observed in Fig. 1, in passing from one method to the other, peaks 12–13, 16–17–18, 20–21 and 27–28 undergo different grades of coelution. These are substances that have only slight chemical differences and have, in traditional analysis applications, a near to baseline resolution. Fig. 2 illustrates a 7 min and 18 s interval of the chromatograms seen in Fig. 1. As it can be seen, the

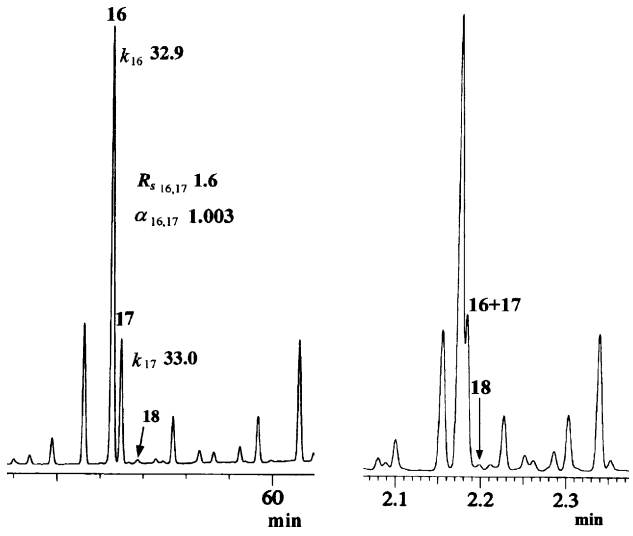

Fig. 2. Two expanded sections of the chromatograms shown in Fig. 1.

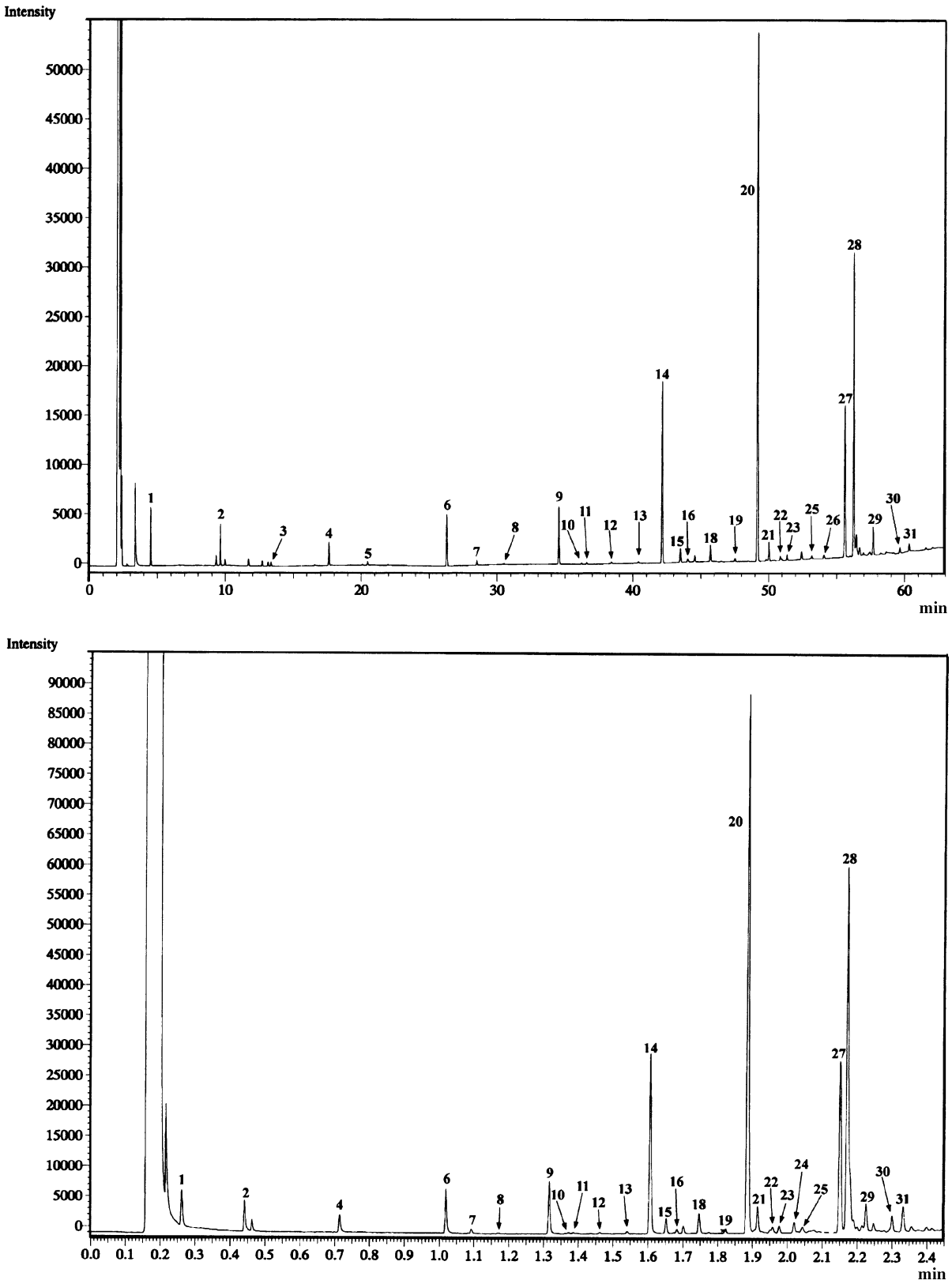

Fig. 3. Conventional and fast GC chromatograms of butter.

Table 1
Conventional and fast GC retention times of the menhaden oil FAMEs

Peak	Compound	Conventional			Fast		
		X	*s*	R.S.D. (%)	*X*	*s*	R.S.D. (%)
1	$C_{14:0}$	42.188	0.004	0.010	1.607	0.002	0.130
2	$C_{15:0anteiso}$	44.043	0.005	0.011	1.653	0.002	0.105
3	$C_{15:0iso}$	44.563	0.005	0.012	1.683	0.002	0.124
4	$C_{15:0}$	45.725	0.005	0.010	1.702	0.003	0.155
5	$C_{16:0iso}$	47.529	0.006	0.013	1.747	0.002	0.119
6	$C_{16:0}$	49.242	0.006	0.012	1.823	0.002	0.114
7	$C_{16:1\omega7}$	50.087	0.006	0.012	1.890	0.002	0.110
8	$C_{16:1\omega5}$	50.458	0.006	0.012	1.918	0.002	0.109
9	$C_{16:2\omega4}$	51.987	0.007	0.013	1.932	0.002	0.108
9	$C_{17:0iso}$	51.987	0.007	0.013	1.932	0.002	0.108
9	$C_{17:0anteiso}$	51.987	0.007	0.013	1.932	0.002	0.108
10	$C_{17:0}$	52.439	0.006	0.012	2.004	0.002	0.105
11	$C_{16:3\omega4}$	53.077	0.007	0.013	2.028	0.002	0.103
12	$C_{17:1}$	54.000	0.007	0.013	2.080	0.003	0.110
13	$C_{18:0iso}$	54.371	0.004	0.010	2.080	0.003	0.110
14	$C_{16:4\omega1}$	54.891	0.006	0.011	2.098	0.002	0.099
15	$C_{18:0}$	55.632	0.007	0.013	2.152	0.003	0.123
16	$C_{18:1\omega9}$	56.294	0.008	0.013	2.173	0.003	0.122
17	$C_{18:1\omega7}$	56.492	0.008	0.013	2.173	0.003	0.122
18	$C_{18:1\omega5}$	56.868	0.006	0.011	2.173	0.003	0.122
19	$C_{18:2\omega6}$	57.694	0.008	0.013	2.225	0.002	0.094
20	$C_{18:2\omega4}$	58.314	0.007	0.012	2.249	0.002	0.103
21	$C_{18:3\omega6}$	58.646	0.007	0.012	2.249	0.002	0.103
22	$C_{18:3\omega4}$	59.249	0.008	0.014	2.284	0.002	0.091
23	$C_{18:3\omega3}$	59.666	0.008	0.013	2.301	0.002	0.090
24	$C_{18:4\omega3}$	60.620	0.008	0.013	2.337	0.002	0.089
25	$C_{18:4\omega1}$	60.955	0.008	0.013	2.350	0.003	0.113
26	$C_{20:0}$	61.584	0.008	0.012	2.398	0.003	0.110
27	$C_{20:1\omega9}$	62.208	0.009	0.014	2.419	0.003	0.109
28	$C_{20:1\omega7}$	62.448	0.009	0.014	2.419	0.003	0.109
29	$C_{20:2\omega6}$	63.084	0.007	0.012	2.454	0.002	0.102
30	$C_{20:2\omega4}$	63.588	0.008	0.012	2.474	0.003	0.122
31	$C_{20:3\omega6}$	64.412	0.007	0.011	2.500	0.003	0.106
32	$C_{20:4\omega6}$	65.079	0.008	0.012	2.525	0.003	0.105
33	$C_{20:3\omega3}$	65.478	0.012	0.019	2.543	0.003	0.104
34	$C_{20:4\omega3}$	66.274	0.008	0.012	2.573	0.003	0.103
35	$C_{20:5\omega3}$	66.982	0.008	0.011	2.600	0.003	0.102
36	$C_{21:5\omega3}$	69.838	0.009	0.012	2.720	0.002	0.077
37	$C_{22:5\omega6}$	71.35	0.008	0.012	2.792	0.003	0.150
38	$C_{22:4\omega3}$	71.65	0.010	0.013	2.806	0.003	0.130
39	$C_{22:5\omega3}$	72.367	0.010	0.013	2.832	0.003	0.093
40	$C_{22:6\omega3}$	73.169	0.011	0.015	2.865	0.002	0.081

fast application does not achieve the separation of $C_{18:1\omega9}$ and $C_{18:1\omega7}$ FAMEs, whereas the same compounds separate with sufficient resolution in the conventional application. In all other cases, the minimal separation observed is enough to allow peak identification and quantitation. Peak shapes and symmetry were satisfactory in both applications as can be more easily observed in Fig. 2. R_s values were calculated for other three pairs of compounds that covered most of the two chromatographic elution ranges with decreases between 70 and 80% (Table 2). Relative quantities were also calculated with limited variations observed (Table 3). Some minor peaks (<1% relative quantity) were characterized by fluctuations as can be seen from the standard deviation values and R.S.D. relative area data, while larger peaks were not affected by this factor; in fact, in this case, the 3% mark for R.S.D. was never passed. The aforementioned doubts towards the possibility of a lack of reproducibility concerning fast data (retention times and relative area %) proved to be, at this point, unfounded. As regards the butter sample,

Table 2
Resolution values and percentage decrease of three peak pairs in the menhaden oil sample chromatograms

Peaks	R_s (conventional)	R_s (fast)	Decrease (%)
6–7	6.145	1.666	72.88
23–24	8.904	1.800	79.78
39–40	5.621	1.375	75.53

characterized by the presence of low-molecular-mass FAMEs ($C_{4:0}$–$C_{10:0}$), there was a certain reduction in resolution but no overlapping of critical peaks occurred. The identification of 31 and 28 compounds was obtained respectively with conventional and fast methods (Fig. 3). The loss of three minor peaks ($C_{7:0}$, $C_{9:0}$, $C_{18:0iso}$) in this case, was due to the low quantity of sample injected. Table 4 reports quantitative data relative to this and the other samples analyzed. As it can be seen, the same observations made for menhaden oil can be extended to these samples. All fast GC chromatograms were characterized by symettric, narrow, and high peak shapes giving no signs of column overloading.

The GC instrumentation employed proved to be reliable in the fast application as it easily satisfied experimental requirements such as rapid heating or high inlet pressures (414.5 kPa). Furthermore, initial doubts concerning peak detection were erased. The detection system was set at a sampling frequency of 50 Hz. For accurate integration, it is known that about 10 data points for just over peak half width are required [20]. The $C_{14:0}$ FAME (peak 1) in the menhaden oil fast chromatogram, for example, has a base width of 0.45 s corresponding to a width at half height of 0.29 s. The 50 Hz sampling rate, in this case, supplied about 22.7 data points which were more than sufficient.

One of the major drawbacks of fast GC techniques, as was discussed before, is a limited sample capacity which can cause the lack of detection of minor quantity peaks and sets a limit on diameter reductions. Although modern instrumentation reduces the risk of band broadening, due to column overloading, with highly controlled split flows, one of the objects of this research was to define the correlation between solute quantity and efficiency. Theoretical plate numbers in function of sample quantities were evaluated on both types of columns employed in this work. A $C_{16:O}$ methyl ester was used in conventional and fast applications at equal isothermal temperatures while all other experimental conditions were unaltered. The results relative to both columns are shown graphically in Fig. 4. As it can be seen, the maximum theoretical plate number is constant up until a threshold amount of sample. It was determined that for the conventional column, quantities of up to 50 ng can be accommodated without affecting resolving power, while for the 0.1 mm I.D. column the same is true for values below 1 ng. The presence of higher solute quantities, in both cases, caused substantial peak distortion.

All rapid analyses are characterized by the application of higher than optimum linear velocities and a consequential decrease in efficiency. Open-tubular column band broadening is described by the classical Golay–Giddings equation [21–24]. As such, good height equivalent to one theoretical plate estimates can be attained for a specific solute in determinate analytical conditions. A series of studies concerning the relationship between column efficiency and gas velocity have been reported in the literature [25,26]. It is well known, that for columns with a high phase ratio it is the resistance to mass transfer in the gas phase which controls

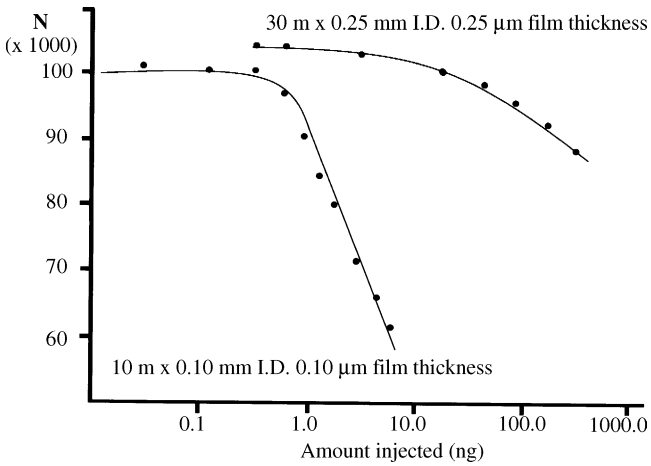

Fig. 4. Sample capacity relative to a micro-bore (0.10 mm) and a conventional (0.25 mm) column ($C_{16:0}$ methyl ester run at 150 °C).

H and consequently the stationary phase contribution to band broadening can be neglected [27]. In this case, H_{min} can be considered approximated to the internal column diameter value especially for higher k values (10 or more). In order to verify the correspondence between theoretical and experimental values, Van Deemter curves (derived from experimental data) were drawn for the conventional and micro-bore capillary column employed in this work (Fig. 5). From the observation of the fast Van Deemter curve it can be seen that H_{min} (0.128 mm), which refers both to column and extra-column band broadening, is in good agreement with theoretical dictations. Moreover, the application of higher linear velocities causes a gradual increase in plate heights as can be expected for a hydrogen mobile phase. The calculated plate number for H_{min} is 78125 while that calculated for the linear velocity (119.9 cm/s) employed in the rapid applications is approximately 30670. The loss in resolution, discussed before, is the consequence of the application of a linear velocity that is more than twice its optimum value (around the 50 cm/s mark). The conventional Van Deemter curve is characterized by an H_{min} value of 0.278 mm (N = 107883) at an average linear velocity of 30 cm/s which is also in good agreement with chromatographic theory. The experimental determination of the above correlations is particularly useful for method optimization as theoretical calculations give, frequently, approximative values.

Reliable peak identification was achieved through the combination of different information sources such as GC–MS spectra and linear retention indices contained in a laboratory constructed library [28–30] and GC × GC. Doubts, that initially arose, about the capability of the MS detection system to produce sufficient spectra at the above described operational conditions, proved to be unfounded (Fig. 6). The quadrupole mass spectrometer, operated at a scan speed of 10,000 amu/s and at a mass range of 50–350 m/z, supplied 25 spectra/s, that were more than sufficient for peak assignment. This can be observed in Fig. 7 which

Table 3
Conventional and fast GC relative area % of the menhaden oil FAMEs

Peak	Compound	Conventional			Fast		
		X	*s*	R.S.D. (%)	*X*	*s*	R.S.D. (%)
1	$C_{14:0}$	7.533	0.056	0.739	7.570	0.026	0.377
2	$C_{15:0iso}$	0.252	0.002	0.677	0.255	0.005	1.859
3	$C_{15:0anteiso}$	0.077	0.001	0.919	0.079	0.001	0.608
4	$C_{15:0}$	0.470	0.003	0.667	0.475	0.013	2.463
5	$C_{16:0iso}$	0.096	0.001	0.658	0.100	0.006	4.505
6	$C_{16:0}$	22.721	0.133	0.585	22.796	0.085	0.430
7	$C_{16:1\omega7}$	10.227	0.053	0.515	10.310	0.036	0.358
8	$C_{16:1\omega5}$	0.287	0.003	0.931	0.291	0.021	4.987
9	$C_{16:2\omega4}$	1.296	0.006	0.472	1.301	0.018	1.172
9	$C_{17:0iso}$	–	–	–	–	–	–
9	$C_{17:0anteiso}$	–	–	–	–	–	–
10	$C_{17:0}$	0.413	0.009	2.101	0.401	0.011	3.243
11	$C_{16:3\omega4}$	1.353	0.021	1.541	1.360	0.025	1.270
12	C17:1	0.156	0.004	2.316	0.360	0.022	5.156
13	C18:0iso	0.215	0.004	1.987	–	–	–
14	$C_{16:4\omega1}$	0.612	0.004	0.594	0.616	0.003	0.415
15	$C_{18:0}$	3.195	0.012	0.384	3.202	0.002	0.047
16	$C_{18:1\omega9}$	10.110	0.046	0.456	12.936	0.042	0.323
17	$C_{18:1\omega7}$	2.639	0.012	0.448	–	–	–
18	$C_{18:1\omega5}$	0.110	0.000	0.192	–	–	–
19	$C_{18:2\omega6}$	1.062	0.004	0.403	1.088	0.009	0.796
20	$C_{18:2\omega4}$	0.343	0.002	0.657	0.375	0.021	3.098
21	$C_{18:3\omega6}$	0.250	0.007	2.604	–	–	–
22	$C_{18:3\omega4}$	0.356	0.007	1.882	0.364	0.014	2.912
23	$C_{18:3\omega3}$	1.080	0.005	0.425	1.089	0.014	1.097
24	$C_{18:4\omega3}$	2.804	0.042	1.504	2.841	0.018	0.610
25	$C_{18:4\omega1}$	0.202	0.000	0.244	0.209	0.005	2.071
26	$C_{20:0}$	0.185	0.001	0.455	0.178	0.011	3.296
27	$C_{20:1\omega9}$	1.393	0.011	0.755	1.551	0.012	0.676
28	$C_{20:1\omega7}$	0.217	0.007	3.145	–	–	–
29	$C_{20:2\omega6}$	0.246	0.001	0.604	0.256	0.004	1.431
30	$C_{20:2\omega4}$	0.156	0.003	2.066	0.160	0.010	5.363
31	$C_{20:3\omega6}$	0.357	0.020	5.558	0.339	0.006	2.669
32	$C_{20:4\omega6}$	0.647	0.003	0.514	0.667	0.005	0.619
33	$C_{20:3\omega3}$	0.159	0.004	2.387	0.161	0.005	3.166
34	$C_{20:4\omega3}$	1.504	0.006	0.376	1.550	0.018	1.189
35	$C_{20:5\omega3}$	12.396	0.046	0.369	12.390	0.048	0.401
36	$C_{21:5\omega3}$	0.899	0.013	1.429	0.861	0.026	4.116
37	$C_{22:5\omega6}$	0.304	0.006	1.999	0.331	0.034	5.374
38	$C_{22:4\omega3}$	0.129	0.003	2.200	0.165	0.009	3.525
39	$C_{22:5\omega3}$	2.035	0.021	1.037	2.069	0.074	2.980
40	$C_{22:6\omega3}$	11.513	0.258	2.241	11.236	0.305	2.434

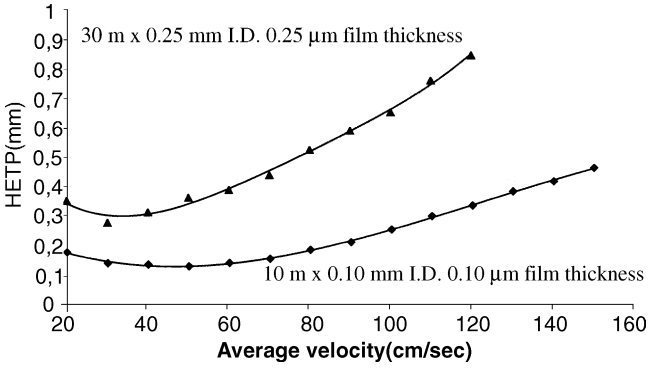

Fig. 5. Conventional and fast Van Deemter curves ($C_{16:0}$ FAME run at 150 °C).

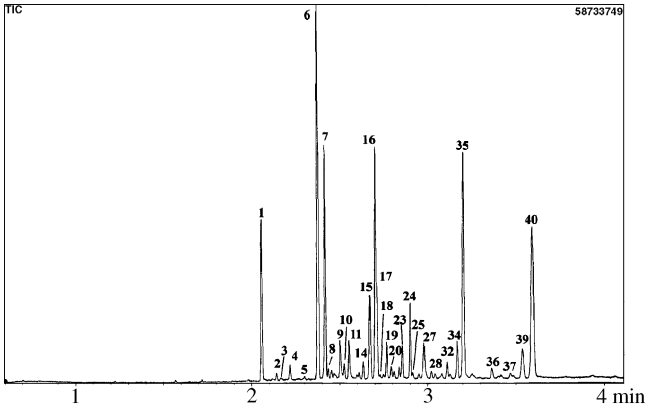

Fig. 6. Fast GC–MS chromatogram of menhaden oil.

Table 4
Relative area % of all fat and oil FAMEs

Compound	Butter (conventional/fast), area %	Peanut oil (conventional/fast), area %	Corn oil (conventional/fast), area %	Sunflower oil (conventional/fast), area %	Soya oil (conventional/fast), area %	Lard (conventional/fast), area %	Tallow (conventional/fast), area %	Olive oil (conventional/fast), area %
$C_{4:0}$	2.09/2.10							
$C_{6:0}$	2.03/1.99							
$C_{7:0}$	0.23/–							
$C_{8:0}$	1.34/1.30							
$C_{9:0}$	0.17/–							
$C_{10:0}$	3.18/3.22					0.07/0.06		
$C_{10:1}$	0.29/0.32							
$C_{11:0}$	0.07/0.09							
$C_{12:0}$	3.61/3.58					0.06/0.06		
$C_{12:1}$	0.09/0.08							
$C_{12:1iso}$	0.11/0.12							
$C_{13:0}$	0.09/0.08							
$C_{14:0iso}$	0.12/0.13							
$C_{14:0}$	11.37/11.39				0.06/0.07	1.16/1.18	3.06/3.18	
$C_{14:1\omega5}$	0.85/0.86						0.77/0.71	
$C_{15:0iso}$	0.23/0.28						0.21/0.18	
$C_{15:0ante.}$	0.45/0.48						0.25/0.26	
$C_{15:0}$	1.05/1.07						0.41/0.39	
$C_{16:0iso}$	0.19/0.22						0.21/0.21	
$C_{16:0}$	35.25/35.32	10.07/10.26	9.84/10.09	5.13/5.44	9.79/10.01	23.75/23.67	28.11/28.29	10.28/10.23
$C_{16:1\omega7}$	1.30/1.28		0.08/0.07	0.07/0.08	0.06/0.05	1.92/1.87	2.65/2.72	0.62/0.63
$C_{17:0iso}$	0.26/0.24						0.42/0.46	
$C_{17:0ante.}$	0.38/0.38						0.94/0.91	
$C_{17:0}$	0.50/0.52		0.05/0.05		0.07/0.08	0.28/0.29	1.13/1.120	
$C_{17:1}$	0.24/0.26				0.05/0.06	0.28/0.27	0.69/0.67	
$C_{18:0iso}$	0.08/-						0.42/0.43	
$C_{18:0}$	10.14/10.38	3.06/3.16	1.60/1.58	3.06/3.08	3.63/3.49	13.44/13.57	17.61/17.58	2.40/2.42
$C_{18:1\omega9}$	21.19/21.13	65.01/65.31	26.30/26.93	27.30/28.93	21.05/21.31	45.43/45.31	40.70/40.14	77.97/78.17
$C_{18:1\omega7}$		0.47/–	0.44/–	0.44/–				1.59/–
$C_{18:2\omega6}$	2.20/2.30	16.35/15.99	60.26/59.90	62.97/61.30	58.74/57.73	11.37/11.25	2.01/2.14	5.41/5.68
$C_{18:3\omega3}$	0.40/0.42		0.74/0.70	0.06/0.08	5.61/5.50	0.61/0.62		0.60/0.59
$C_{18:2con.}$	0.51/0.49						0.40/0.42	
$C_{20:0}$		1.56/1.53	0.44/0.42	0.21/0.20	0.39/0.37	0.24/0.27		1.12/1.10
$C_{20:1\omega7}$		1.09/1.08	0.25/0.26	0.18/0.18	0.15/0.16	0.71/0.72		
$C_{20:3\omega6}$						0.39/0.33		
$C_{20:3\omega3}$						0.29/0.28		
$C_{22:0}$		1.56/1.62		0.59/0.66	0.50/0.48			
$C_{24:0}$		0.82/0.84						

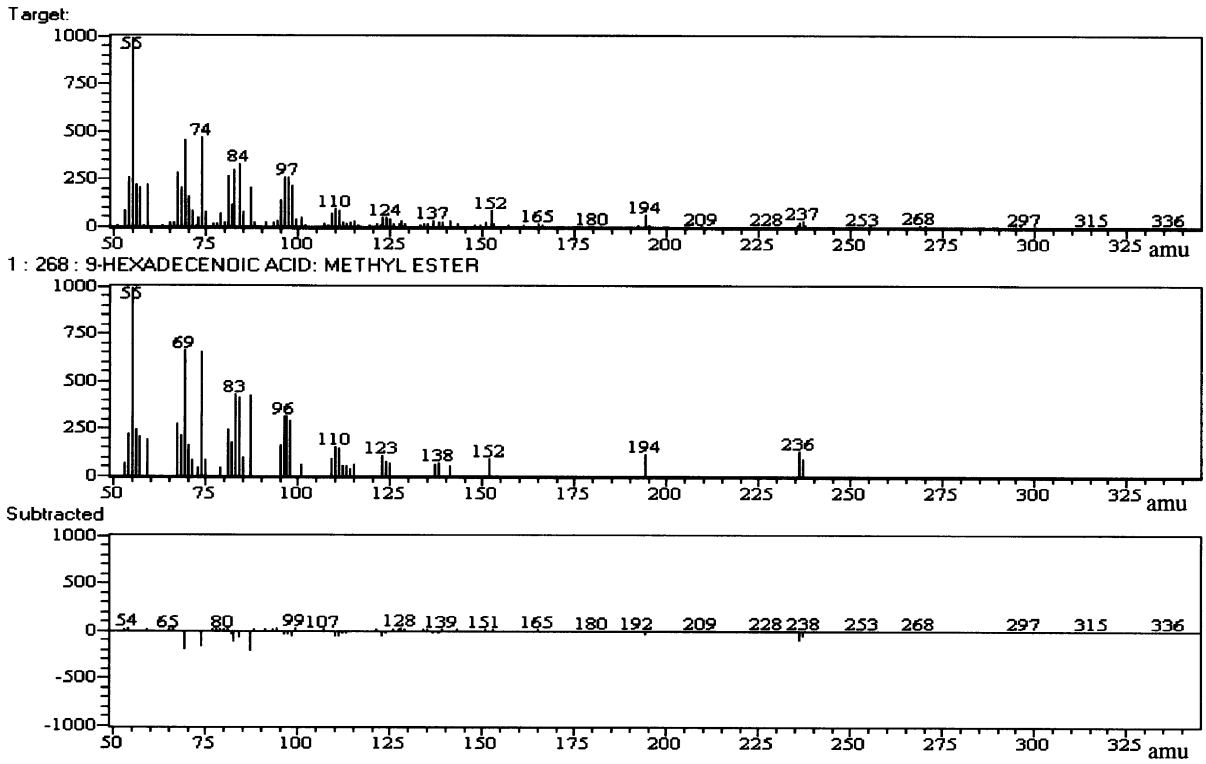

Fig. 7. Comparison between fast GC–MS and library spectra relative to $C_{16:1\omega7}$ FAME.

illustrates a comparison between fast GC–MS spectra ($C_{16:1\omega7}$) and that provided by a commercial library (Wiley and NIST): no relevant difference can be observed.

4. Conclusions

Modern GC separations are converging towards the combination of high speed and high resolution. The above investigation is an attempt to define the boundaries in which speed and analytical quality can be confined. Although the passage to fast GC saw the application of conditions that caused an unavoidable decrease in efficiency, the overall analytical result was quite good and an over 95% reduction in analysis time was achieved. There is obviously more room for method optimization and development, which will also concern the application of ultimate generation micro-bore columns with a higher resolving power than those used in this research. In fact, the aim of future investigations will be that of improving the fast chromatographic performance as regards the separation of critical compounds while maintaining the same speed as seen in this work. This technique proved to be applicable on complex lipidic samples and can certainly be extended to other matrices. Another important aspect that must be again mentioned is the exploitation of comprehensive bidimensional GC in combination with other information sources for reliable and thorough peak identification.

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References

[1] K. Asplund, J. Int. Med. 251 (2002) 372.
[2] L. Hooper, J. Hum. Nutr. Diet. 14 (2001) 297.
[3] F.M. Sacks, M. Katan, Am. J. Med. 113(9B) (2002) 13.
[4] K. Eder, J. Chromatogr. B 671 (1995) 113.
[5] H.M. McNair, G.L. Reed, J. Microcol. Sep. 12 (2000) 351.
[6] L.M. Blumberg, J. High Resolut. Chromatogr. 22 (1999) 213.
[7] M. Van Deursen, J. Beens, C.A. Cramers, H-G. Janssen, J. High Resolut. Chromatogr. 22 (1999) 509.
[8] C.A. Cramers, P.A. Leclercq, J. Chromatogr. A 842 (1999) 3.
[9] L. Mondello, A. Casilli, P.Q. Tranchida, L. Cicero, P. Dugo, G. Dugo, J. Agric. Food Chem. 51 (2003) 5602.
[10] L. Mondello, R. Shellie, A. Casilli, P.Q. Tranchida, P. Marriott, G. Dugo, J. Sep. Sci. (2004) in press.
[11] A. van Es, High Speed Narrow Bore Capillary Gas Chromatography, Hüthig, Heidelberg, 1992.
[12] V. Jain, J.B. Phillips, J. Chromatogr. Sci. 33 (1995) 601.
[13] D.H. Desty, A. Goldup, W.T. Swanton, in: N. Brenner, J.E. Callen, M.D. Weis (Eds.), Gas Chromatography, Academic Press, New York, 1962, p. 105.
[14] E. Matisovà, M. Dömötörovà, J. Chromatogr. A 1000 (2003) 199.
[15] M.M. van Deursen, J. Beens, H.-G. Janssen, P.A. Leclercq, C.A. Cramers, J. Chromatogr. A 878 (2000) 205.
[16] K. Mastovskà, S.J. Lehotay, J. Chromatogr. A 1000 (2003) 153.
[17] P. Korytár, H.-G. Janssen, E. Matisová, U.A.Th. Brinkman, Trends Anal. Chem. 21 (2002) 558.

[18] S. Dagan, A. Amirav, J. Am. Soc. Mass Spectrom. 7 (1996) 737.
[19] L. Mondello, A. Casilli, P.Q. Tranchida, P. Dugo, G. Dugo, J. Chromatogr. A 1019 (2003) 187.
[20] J.V. Hinshaw, LC–GC Eur. 15 (2002) 152.
[21] M.J.E. Golay, in: D.H. Desty (Ed.), Gas Chromatography, Butterworths, London, 1958.
[22] G.H. Stewart, S.L. Seager, J.C. Giddings, Anal. Chem. 31 (1959) 1738.
[23] J.C. Giddings, S.L. Seager, L.R. Stucki, G.H. Stewart, Anal. Chem. 32 (1960) 867.
[24] C.A. Cramers, C.E. van Tilburg, C.P.M. Schutjes, J.A. Rijks, G.A. Rutten, R. De Nijs, in: J.A. Rijks (Ed.), Proceedings of the Fifth Symposium on Capillary Chromatography, Riva del Garda, 26–28 April 1983, Elsevier, Amsterdam, 1983, p. 76.
[25] G. Gasper, R. Annino, C. Vidal-Madjar, G. Guiochon, Anal. Chem. 50 (1978) 1512.
[26] L.M. Blumberg, J. High Resolut. Chromatogr. 20 (1997) 597.
[27] P. Sandra, C. Bicchi (Eds.), Capillary Gas Chromatography in Essential Oil Analysis Hüthig, Heidelberg, 1987, p. 38.
[28] H. Van Den Dool, P.D. Kratz, J. Chromatogr. 11 (1963) 463.
[29] G. Tarjan, J. Chromatogr. 472 (1989) 1.
[30] L. Mondello, P. Dugo, A. Basile, G. Dugo, J. Microcol. Sep. 7 (1995) 581.