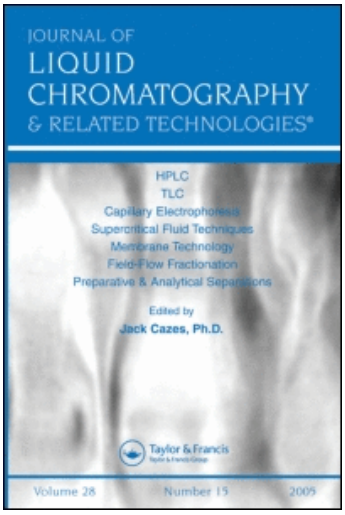

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Recent Advances in Fast Gas-Chromatography: Application to the Separation of Fatty Acid Methyl Esters

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To cite this Article Cruz-Hernandez, Cristina and Destaillats, Frédéric(2009) 'Recent Advances in Fast Gas-Chromatography: Application to the Separation of Fatty Acid Methyl Esters', Journal of Liquid Chromatography & Related Technologies, 32: 11, 1672 — 1688
To link to this Article: DOI: 10.1080/10826070902956386
URL: http://dx.doi.org/10.1080/10826070902956386

Journal of Liquid Chromatography & Related Technologies®, 32: 1672–1688, 2009

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826070902956386

Recent Advances in Fast Gas-Chromatography: Application to the Separation of Fatty Acid Methyl Esters

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Abstract: Gas-chromatography (GC) is a powerful separation technique for resolving and quantifying a wide range of compounds, such as fatty acid methyl esters (FAME) derivatives. Separation of common FAME is typically achieved on highly polar or polyethylene glycol stationary phases that can separate fatty acids according to chain length, number and geometry of ethylenic double bonds. GC is one of the most commonly available methods for fatty acid analysis with constant improvements for different applications. One such improvement is faster GC analysis, which has long been the focus of investigators researching the application and benefits of this technique. A greater speed of analysis offers many key benefits, such as increased sample throughput, reduced analytical expenses, and increased laboratory productivity. Fast GC analysis can be accomplished by using a short column with reduced column film thickness, high carrier gas velocity, and fast program temperatures. A summary of options for fast GC analysis with emphasis on FAME analysis is discussed in the present review.

Keywords: Fast analysis, Fatty acid methyl ester, Gas chromatography, Lipid

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INTRODUCTION

Although an ongoing interest has been to improve GC separation speed since the early 1960s, it was not until the last decade that some approaches were proposed and applied to routine analysis. The last 10 years have seen the introduction of electronic pressure control of the mobile phase, high frequency FID detectors, and time of flight mass spectroscopy to detect high speed eluting peaks, as well as the software to facilitate method revalidation and data treatment. Many efforts have been undertaken towards designing automatic GC and to speed up analysis times, focusing in many cases on the fundamentals of the partitioning process in the chromatographic column, considering that such applications can be challenging for automatic control of chemical processes.

Since the introduction of GC in 1952, it has been an ongoing interest in improving separation speed and having a better control of the analysis. In the late 1950s, Ayers and De Ford proposed an optimized operational parameters of chromatograms where a group of six hydrocarbons were analyzed in 25 seconds.[1] Temperature programming has subsequently been used to speed analysis of wide boiling range samples by controlling the column temperature.[2] Simultaneously, it was recognized that, as with heat, cooling a column faster also resulted in more rapid chromatographic analysis. Perkin-Elmer introduced a GC in 1961 equipped with direct or resistive column heaters. A low voltage, high amperage current is passed through the column, which is heated by resistance heating; this enables the column to act as its own heating element.[3]

This early recognition of instrumentation and methodology, include all the external hardware parameters, which control the retention times of samples as they pass through the chromatographic column.[4] Meanwhile, the introduction of tubular columns by Golay in 1957, were rapidly recognized for their benefits on resolution and speed of analysis.[5] Capillary columns with a reduced diameter for high speed separation were later proposed by Desty and co-workers in 1962.[6] In 1979 Dandeneau and Zerenner introduced the fused capillary columns and demonstrated that once they are coupled to a GC system, transition from packed to capillary columns is improved. It was at the same time in the 1960s that analyst often used columns, which were much longer than necessary.[7] These researchers were more worried about how to solve the problem of separation and identification of complex mixtures than to perform faster analyses.

Improving GC equipment has long been a consideration, and it was in the 1980s that the microhip GC was introduced by Microsensor technology (Fremont, CA), providing faster analysis through miniaturization of each of the chromatographic components. Some drawbacks (i.e., no

backflush, limited column/detector choices, lack of temperature control), affected its usefulness for fast analysis, however. In the 1990s, interest in decreasing the time of analyses has grown significantly due to the increase of cost per analysis (including equipment cost), growth in the number of samples requiring analysis, as well as shorter analyses time available within laboratories and companies. The multicapillary column was introduced in the 1990s by Alltech, combining over 900 liquid phase coated, 40 μm capillaries in a single glass tube in order to provide significantly shorter analysis.[3] In 1991, Thermedics Detection, Inc. (Chelmsford, MA) developed a very fast temperature programming technique using resistive heating of a fused silica capillary column contained within a metal tube.[8] Thomas and Bennett, patented a chromatographic system for fast GC analyses with a micro packed column and inner and outer jacket tubes with air between the two jackets in 2002.[9] Air also flows in the enclosure to keep the heat from the valve and the FID from heating the column. Many chromatographic systems have been introduced in the market. In some way, each has demonstrated the potential for being adapted for fast GC analysis, their characteristics will be reviewed.

TERMINOLOGY: FROM CONVENTIONAL TO ULTRAFAST

The need for faster analysis times for GC has produced different approaches to speed up the normal analysis. These different solutions have produced discussion on what can truly be considered "high speed", "fast GC", "ultra-fast GC", etc. Such classification might not be academic but can be very helpful with regard to the requirements of the instruments to be used. In general, fast GC has been defined as the analysis of a sample by GC in a shorter period than what conventional GC methods required in order to separate the same compounds.[10,11]

Faster GC is classified based on the peak widths obtained.[12–14] Such classification is based on the consideration that every reduction of analysis time results in an identical reduction in the widening of the chromatographic zone due to the shorter residence time of the components in the column.

1. Fast GC analysis with separation in the range of minutes and the average peak width between 1–3 s. Fast GC is most commonly used in faster chromatography applications.

2. Very Fast GC analysis with separation in the range of seconds and the average peak width between 30–200 ms.

3. Ultra Fast-GC analysis with separation in the sub-second range and the average peak width between 5–30 ms. Simple separations can be performed by very fast and ultra-fast GC, which are less commonly used for faster chromatography.

There is also a similar, yet more specific definition for Fast GC,[15] which would be analysis with separation performed in less than 10 min with columns with I.D. between 0.25 and 0.1 mm, length from 5 to 15 m, temperature programming rates of 20–60°C/min and peak widths in the range between 0.5–2s. Ultra fast GC is defined as one with separation in 1 min or less, entailing the use of short (2 – 10 m) narrow-bore columns (0.1–0.05 mm I.D.) and temperature programming rates above 1°C/s, leading to peak widths of 50–200 ms, as peaks of less than 10 ms are at present difficult to obtain in practice. A classification can be very helpful when it comes to choose the appropriate instrumentation but it will also depend on the application in turn.

GC PERFORMANCE CHARACTERIZATION AND OPTIMIZATION

In order to decide which approach will be taken to increase the separation speed in capillary GC, the initial consideration must be the characteristics of the sample to be analyzed. Not all applications are the same and ultimately a complete separation of the compounds in the mixture should be one's target.

In many cases the plate number (N) of the capillary column is too high for a given separation problem, resulting in solutes that are not well separated and offer impaired resolution. Typical ways to shorten the analysis time in this case are to either decrease the column length or to increase the carrier gas flow rate to a point far above the optimum. Recent alternatives include the use of multi-channel columns. Unfortunately, there is no single method that will result in a significant time reduction for all applications; however, sometimes more than one route can be adopted and each will eventually produce the same results. Following are listed the most relevant parameters towards a faster separation GC method.

Columns

Short columns with conventional or reduced I.D. coated with a suitable stationary phase have been shown to be effective in reducing analysis time for different samples and to be complementary to narrow-bore columns for fast GC. Two parameters can be modified easily by the analyst and offer noticeable effects on resolution. The column temperature can be adjusted, depending on the compound volatility range. Changes in column length or the use of a higher carrier gas velocity is beneficial to reduce analysis time, although reductions will vary depending on the type of column use.

Conventionally packed columns have been used since the introduction of GC[3]. These columns, containing a low proportion of partitioning agent and a carrier gas of low viscosity and high diffusivity gave a nine fold improvement in the time required to obtain a given chromatographic separation.[16]

Micropacked columns, packed columns with an I.D. of 0.5 to 1 mm, with a particle to column diameter ratio of 1 to 5, have been used in GC since 1963.[17] The use of this type of column for "High Speed GC" was reported by Guillemin in 1977, using a Spherosil column (spherical silica gel beads) that provided a reduction of time analysis by a factor of 2–5 with no changes in hardware. Several parameters such as linear gas velocity, column diameter/length, and particle size can be varied and, therefore, taken into consideration for rapid analysis.[18] The advantages include reproducibility, a small carrier gas flow rate, and high efficiency. The pressure drop is not excessive while the number of theoretical plates per unit length is high. The disadvantages can be that these columns are normally packed in 1 1/16 inch stainless tubing, and it is visually impossible to determine if there are empty spaces within the column, besides the difficulty on packing longer lengths (>10 ft).

The capillary (0.1 to 1.0 mm I.D.) or wall coated open tubular (WCOT) columns were introduced by Golay in 1957. Parameters such as column diameter/length, stationary film thickness, column material, and carrier flow/pressure have been studied and optimized for reduced analysis time in process applications.[19] The wide-bore capillary has been of particular interest for process applications because it can be used as a direct replacement of a packed column, without changing operating parameters or sample preparation. The associated benefit is a significant decrease in analysis time without changing sample size.[20]

Some disadvantages are related with this application such as the critical injection in narrow-bore columns, where peak tailing can easily appear if the dead volumes are not kept to the minimum because of the very low flow in the column (I.D. < 150 um).

Multicapillary columns (usually 1 m long) were introduced to solve the limitation of narrow-bore columns, specifically to increase sample capacity without sacrificing sensitivity. Such columns were made by combining some 900 capillaries of 40 um I.D. in a single glass tube, which maintain high efficiency across a broader flow rate range and operate at lower temperatures, thus providing faster analyses. These columns are appropriate for relatively simple mixtures that require only low plate numbers.[3]

Carrier Gas

Hydrogen provides better efficiency and resolution when used as a carrier gas, one of its many advantages in fast GC. Nowadays GC systems have

better electronics and the possible risk of leaking is much lower than before. Modern GC has the option of the so called gas saver mode and can perform an automated safety shut off of carrier gas if there is a sudden pressure decrease.

At higher carrier gas velocities, the speed of analysis can be improved as long as velocity overrules the required column length increment.[21] In general, the carrier gas velocity of a fast GC separation is 40% above the optimal value only and 8% faster analysis can be obtained.[22,23] By increasing the pressure/flow in a controlled manner, the time for the sample to reach equilibrium is reduced and the sample is swept through the column to the detector by the faster flow of the carrier gas. Pressure/flow can be used independently of temperature, or both can be used simultaneously to speed the analysis. Modern GC allow pressure/flow programming of the carrier gas, which is useful to reduce time, especially when the liquid phase in the column has reached its maximum operating temperature. A benefit to having available electronic pressure control units is that it can be operated up to 10–12 bar advantage that is compatible with most narrow-bore columns.[10]

Injection Systems

The sample injector is a very critical part of the GC equipment and needs to be well designed and well maintained. The simplest injection system for fast GC is the split injector. When temperature programmed conditions are used, is possible to lower split ratios, because the input band can go back to the initial temperature. Band widths in the range of milliseconds can be obtained using high split flows (around 2l/min) when samples under study do not contain heavy analytes, although the detection limits are not ideal.

Fast GC can also be used on-column or with splitless programmed temperature vaporization injections, although these are less popular injection methods due to their characteristics. Splitless injection, requires an injector liner with a small I.D. in order to obtain acceptable splitless times at the low column flow of a narrow-bore column.[24] On-column injection is possible when using narrow-bore columns coupled with wider-bore retention gap (250 or 320 um I.D.), and dead volumes that are very low. On-column injections for very fast and ultra-fast GC are not possible; consequently, miniaturised mechanical switching valves are preferred instead.

GC Ovens

Raising temperatures is another way to decrease analysis time when combining the numerous advances available by manipulating the column

types and parameters. GC temperature programming has been used to control columns since early 1960.[25] Increases in temperature cause the time spent by the sample in the liquid phase to decreased, which shifts the equilibrium to the gas phase and reduces the time of analysis. A drawback could be the higher elution temperature of the peaks of interest, especially if the linear velocity is not adjusted. Greater benefits can be obtained if not only heating but cooling cycles are considered, together with the allowed maximum heating and cooling rates in the specific system. Cooling/heating rates have to be adapted when additional parameters are used for faster GC.

In general to avoid compromising resolution, the ideal situation is to keep constant the ratio programming rate/column void time (i.e., around 10°C/void time).[14] Cates and co-workers, developed a fast response thermochromatographic capillary column, which has a thin coating of a metallic compound applied to the outer surface. When a current is passed through the column it heats and cools very quickly.[26] Nowadays, there are systems commercially available (i.e., Thermedics Detection) with resistive heating, capillary columns are surrounded by a resistively heated metal tube, which provides fast increments in temperature (up to 1200°C/min) and at the same time cool rapidly (around 300°C to 5°C in less than 30 s).[27,28] Union Carbide received a patent for a Fast Gas Chromatography Method, Apparatus and Applications. This apparatus employs low dead volume fittings, high speed injectors and detectors, a fast temperature program module, and a high speed data acquisition system. The fast temperature programming module can rapidly heat and cool the column as required to achieve analysis of compounds whose boiling points differ by as 250°C in less than two minutes, possibly in less than one minute.[11]

Detectors

The flame ionization detector (FID) is considered one of the most sensitive GC detectors for hydrocarbons, possessing a linear range of 6 or 7 orders of magnitude (10^6 to 10^7) and limits of detection in the low picogram or femtogram range. The FID is the most widely and successfully fast GC, where more peaks are generated per unit of time, together with the high sampling frequency of the detector, to provide enough points across the peak for accurate representation.[29] A fairly high makeup flow rate is recommended to avoid problems between the column exit and the flame, if any. Modern designs are mostly digital and allow data acquisition rates up to 200 Hz, sufficient even for very fast GC.

For halogenated compounds, the electrocapture detector (ECD) is often the detector of choice. Thermal conductivity detectors (TCD) can

be manufactured with extremely low cell volumes; the detectors in field portable GCs are made by etching and have cell volumes of only a few nanolitres.[30] A detector of rapidly growing importance is the MS. It is primarily used for analyte identification/confirmation, but MS detection is also a means of reducing the analysis time, especially if combined with deconvolution techniques. Several types of MS are available for coupling to GC: If faster detection is required, non scanning MSs, such as time-of-flight (TOF) analysers are an alternative.

APPLICATION TO FATTY ACID METHYL ESTERS ANALYSIS

Interest for fast GC analysis of FAME in different edible oils or biological tissues has increased lately. A summary of the most relevant reports published in this area is provided in Table 1. The most recent advance has been the development of the robotic FAME preparation procedure coupled with fast GC analysis in 2008.[31] This methodology provides a sufficient throughput to allow analysis of plasma or blood samples from large clinical studies,[31] and is in fact, an improved version of the original method developed by the same group and published in 2005.[32] Application of this method has been used to investigate polyunsaturated fatty acid status in large cohorts. In this method, the plasma sample is directly treated without prior lipid extraction; the robot carries the reaction, methyl ester extraction, and sample concentration, leaving the sample in a GC auto sampler vial ready for injection for fast GC analysis. Fast GC analysis duration is about 6 min, and authors claimed that this methodology allows analyzing 200 samples per day.

A very fast GC analysis of cod liver oil samples using a 0.1 mm I.D. polar 2 m column segment was reported by Tranchida and coworkers in 2008.[33] Using this set up the authors were able to analyze the sample in 1.45, while with a 10 m column having the same stationary phase the duration of the run was 2 min. Similar performances were obtained by Mondello and coworkers (2006), who separated FAME ranging from lauric (14:0) to docosahexadecenoic (22:6 n-3, DHA) acids in 2 min.[34]

Bondia-Pons and coworkers (2004) accomplished a faster separation with a speed gain of a factor of 5 compared with conventional GC performed in 16 min.[35] A bought mixture of menhaden oil with internal standard was used to set up and validate the method. Plasma samples were later analyzed and 37 FAs were identified in 3.2 min.[36] The same approach was used to analyze plasma phospholipids with a combination of solid phase extraction fast GC techniques where 25 FA were observed in 3.8 min.[36] Mondello and coworkers analyzed different kinds of samples (menhaden oil, butter, lard, tallow, corn, pea-

Table 1. Column/Brand/Time/FA indentified

Reference No	Column	Time of analysis	Carrier gas	P (kPa)	FAME Separated	Sample Analyzed
52	BPX-70 (10 m × 0.1 mm I.D. × 0.1 μm)	~5 min	H_2		~44 different FAME 4:0 to 22:6 n-3	Milk fat, cocoa butter, tuna oil samples
31	DB-FFAP (15 m × 0.1 mm I.D. ×0.1 μm)	~6 min	H_2	344.7 kPa	~22 different FAME, 14:0 to 22:6 n-3	FAME standards. Plasma sample
53	VF-23 ms (15 m × 0.15 mm × 0.15 μm)	~7 min			~19 different FAME, 16:0 to 24:1 n-9	
33	10 m × 0.1 mm I.D. capillaries, 0.1 mm I.D. polar column segment (2 m), very fast GC	~2 min				Cod liver oil
36	VF-23 ms (10 m × 0.10 mm I.D. × 0.1 μm))	~4 min	He	482 kPa	~25 different FAME, 14:0 to 22:6 n-3	Human plasma spike with PC
34	Omegawax 250 (15 m × 0.1 mm I.D. × 0.1 μm)	~2 min	H_2	437.6 kPa	~22 different FAME, 14:0 to 22:6 n-3	Cod liver oil
32	DB-FFAP (15 m × 0.1 mm I.D. × 0.1 μm)	~8 min	H_2	344.7 kPa	~26 different FAME, 14:0 to 22:6 n-3	Plasma
37	Supelcowax-10 (10 m × 0.1 mm I.D. × 0.1 μm)	~3 min	H_2	414.5 kPa	~44 different FAME 4:0 to 22:6 n-3	menhaden oil, Butter, lard, tallow, corn, peanut, olive, colza, sunflower, soya,
35	BPX-70 (10 m × 0.1 mm I.D. × 0.1 μm)	~3 min	He	560.5 kPa	~37 different FAME, 12:0 to 22:6 n-3	Menhaden oil (Supelco-37FA), C13 AS I.S. Human plasma from Sigma Chemical Co
38	Supelcowax-10 (10 m × 0.1 mm I.D. × 0.1 μm)	~13 min	H_2	414.5 kPa	~44 different FAME 4:0 to 22:6 n-3	Butter, lard, tallow, peanut, corn, sunflower, soya, olive, menhaden oil

nut, olive, colza, sunflower, soya) and reported analysis time of about 2.9 min.[37] In their previous fast GC work, the time was reduced significantly by a factor of 5, when conventional GC (~70 min) was compared with Fast GC (15 min), although analysis time was still significantly longer than the method used in 2004.[37,38] A series of FA were identified from all samples analyzed, although no long chain FA were resolved when butter samples were analyzed.[37,38] Fast GC has been also applied to the analysis of the FA composition of essential oils. [15,37–47] human serum,[48–49] and to the quantification of conjugated isomers of linoleic acid in human plasma.[50–51]

Fast GC can be used to separate complex FAME mixtures as usually achieved by conventional GC. Very often, complex FAME mixtures are obtained from animal lipid samples and most obvious cases are ruminant milk, marine oils, or brain lipid extract samples. To illustrate the typical results that can be obtained using fast GC, we fractionated rat brain lipids to separate the 5 main polar lipid fractions: phosphatidylethanolamide (PE), phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylinositol (PI), and sphingomyelin (SM). These lipid fractions have been methylated using convention acid based methods and analyzed by fast GC, as described by Destaillats and Cruz-Hernandez.[52] Separations achieved are provided in Figures 1–3. The use of very polar fast columns allows obtaining the same resolution as conventional 30–60 m length columns. The resolution of FAMEs prepared from the brain phosphatidylethanolamide (PE) fraction gives a

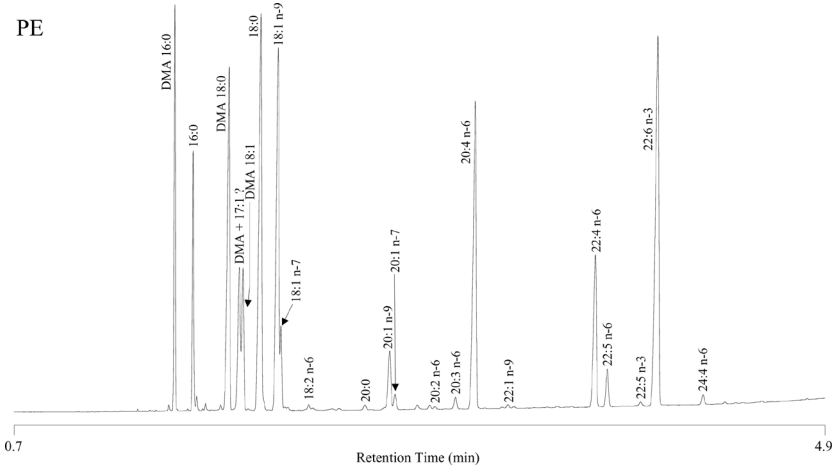

Figure 1. Fast GC trace of fatty methyl esters (FAME) prepared from purified brain phosphatidylethanolamine (PE).

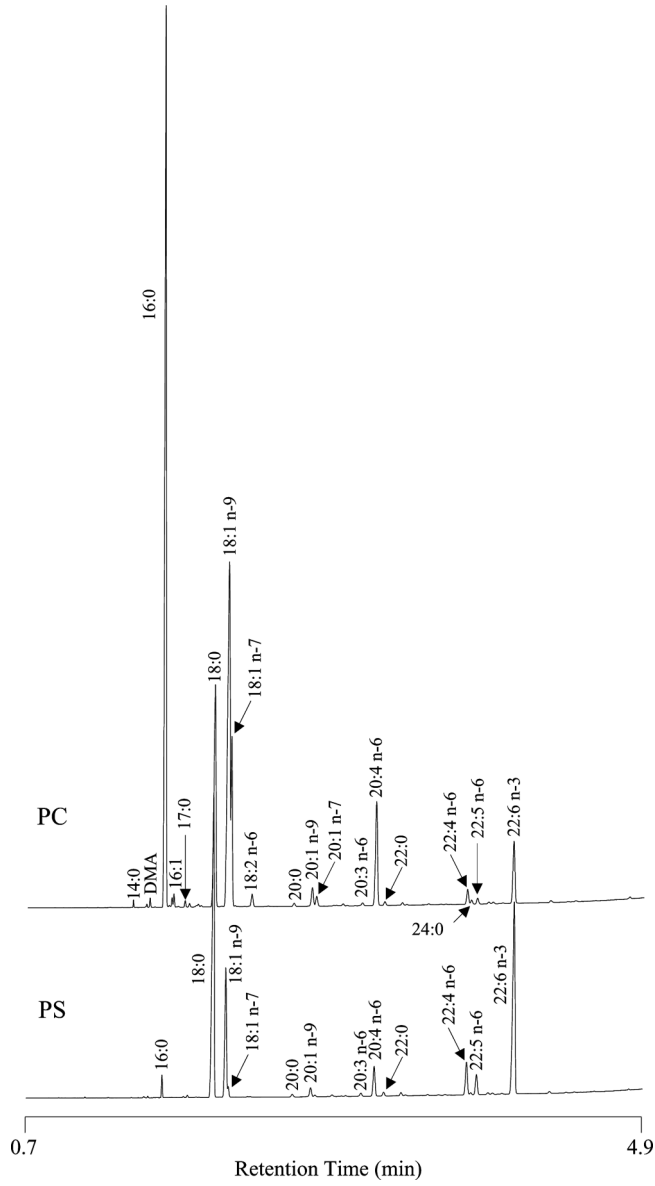

Figure 2. Fast GC trace of fatty acid methyl esters (FAME) prepared from purified brain phosphatidylcholine (PC) and phosphatidylserine (PS).

good example. PE is the most complex phospholipid fraction in brain tissues, due to the occurrence of the plasmenylethanolamides. The resolution of fatty acid methyl esters from PC, and PS are shown in Figure

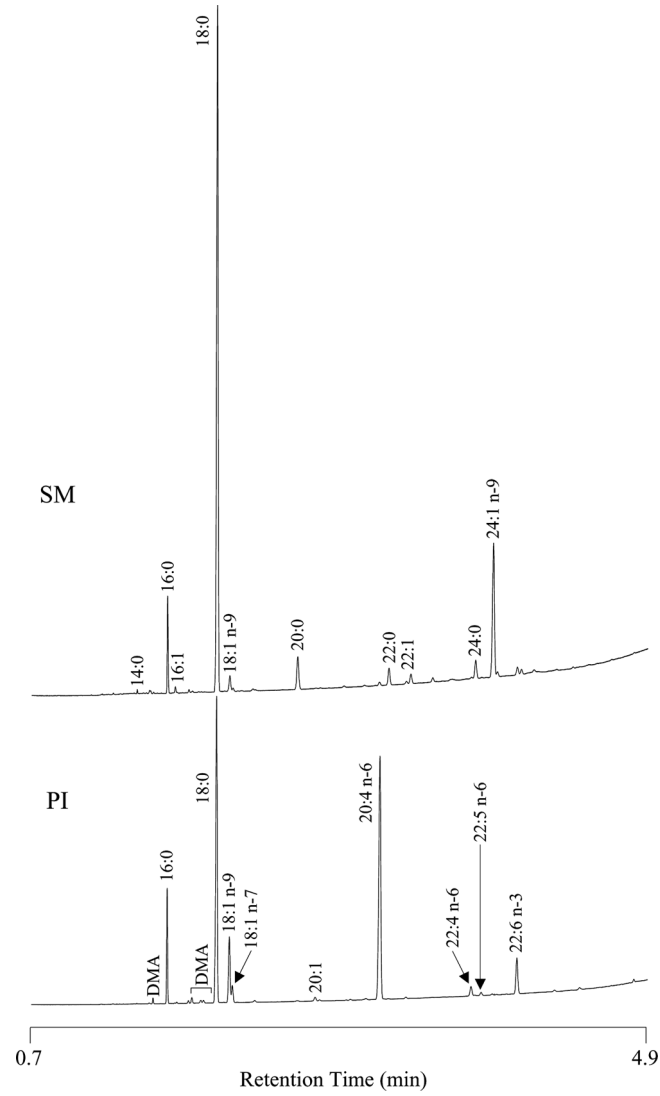

Figure 3. Fast GC trace of fatty acid methyl esters (FAME) prepared from purified brain sphingomyeline (SM) and phosphatidylinositol (PI).

2, and chromatograms of brain SM and PI in Figure 3. The used GC conditions allowed obtaining the full spectra of fatty acids acylated to PE in brain (Figure 1). In addition, the main dimethylacetates (DMA) derivatives formed from plasmenylethanolamide, the main class of brain plasmalogens, are well separated from saturated fatty acids

14:0, 16:0, and 18:0 (Figure 1). As DMAs represent up to 20% of residues found linked to PE in brain, it is very important that operating conditions allow these separations. Resolution of long-chain polyunsaturated fatty acids such as 22:4 n-6, 22:5 n-6, 22:5 n-3, 22:6 n-3, and 24:4 n-6, can be obtained as shown in Figure 1. The main saturated fatty acid found in PC is palmitic acid (Figure 2). Brain PC contains arachidonic (20:4 n-6) and docosahexadecenoic (22:6 n-3) acids, which is the main long chain polyunsaturated fatty acid found in PS (Figure 2). The main saturated fatty acid found in PS, PE, and PI is stearic (18:0) acid (Figures 1–2). In PI, the main long chain polyunsaturated fatty acid found is arachidonic (20:4 n-6) acid (Figure 3).

Fast GC coupled with isotope-ratio mass-spectrometry (IRMS) detection used to analyze FAME and steroids was reported recently.[53] Good peak widths, resolution, and precision were obtained by the authors, which illustrate that fast GC analysis of FAME can be performed using other detectors than FID.

CONCLUSION

Many improvements have been developed in fast GC, whether it is on instrumentation or any component (i.e., columns, injectors, detectors, etc.) that has helped to make the analysis faster without compromising chromatographic resolution and separation. Fast GC columns with reduced I.D. coated with various types of stationary phases are available nowadays, allowing the conversion of almost all the conventional methods. In biological and food samples, lipids always contain a broad range of fatty acids that are conventionally analyzed as methyl ester derivatives by GC on polar columns. The example of fast analysis of FAME presented in the present review showed that fast GC can be used without compromising the quality of the GC separation.

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