A Very Fast Method for the Preparation and GC Analysis of Human Plasma Fatty Acid Methyl Esters

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The major objective of any GC method is the separation of the most critical sample components in the minimum time. This, obviously, becomes of fundamental importance for laboratories with a high sample throughput and/or where there is a need for quick and correct results. As a consequence, there has been an ever-present interest within the chromatographic community for the introduction of faster techniques. The primary aim, relative to any fast GC technique, is to maintain (compared to traditional GC) sufficient resolving power for the separation between the compounds of interest. In respect to this aspect, the narrow-bore column approach is a very efficient way of increasing analysis speed.[1,2] A decrease in column internal diameter reduces resistance to mass transfer in the gaseous phase. Although the use and effectiveness of these columns was demonstrated many years ago, their routine use in fast GC applications is only quite recent. The reason behind this delay is merely technical and was due to the lack of suitable GC equipment. Modern GC systems are now capable of supplying the extreme experimental conditions that narrow-bore columns necessitate: high inlet pressures, highly controlled split flows, rapid oven temperature heating/cooling and fast electronics for detection. It must be added that the sample introduction system is of the highest importance in this type of analytical approach. The employment of a high speed autoinjector is fundamental as it allows the introduction of very narrow sample bands. Furthermore, it also enables the obtainment of highly reproducible retention time data. A further contribution towards the minimization of injection band broadening can be attained through the use of reduced ID inlet liners (i.e., 0.75 mm).

Present-day fast GC applications are generally achieved with 10 m × 0.1 mm ID × 0.1 µm (film thickness) columns. The latter are characterized, approximately, by the same resolving power as a 25 m × 0.25 mm ID × 0.25 µm column (100 000 theoretical plates). Substantial reductions in analyses times are achieved exploiting two factors: a shorter column length and the application of higher than optimum average linear velocities.

The present research is based on the reduction of analysis times in the determination of human plasma fatty acids (FAs). This, is as part of a wider research project characterized by the final aim of greatly reducing plasma FA analyses times and, as a consequence, the cost of clinical assays.

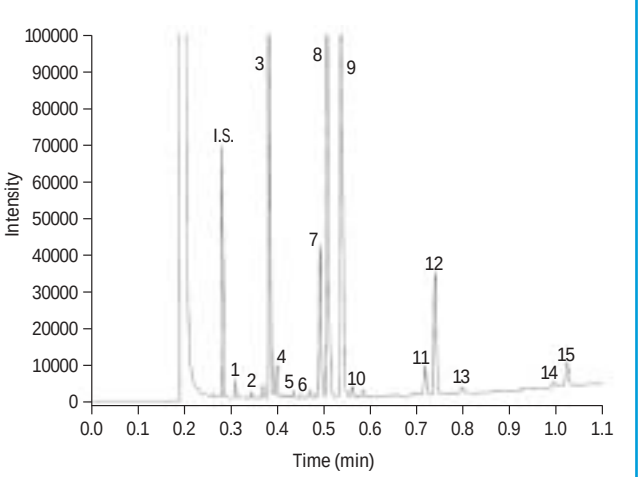
The qualitative/quantitative determination of plasma FAs can be basically divided in two parts: sample preparation and GC analysis. The sample preparation procedure, which consists essentially in plasma FA methylic esterification, has been greatly shortened: 100 µL of plasma were saponified with sodium methoxide at a temperature of 95 °C in 5 min. The subsequent methylic esterification was achieved with boron trifluoride methanol complex at a temperature of 95 °C in 15 min. The fatty acid methyl esters (FAMEs) were extracted in n-hexane.

GC analyses were performed on a Shimadzu GC-2010 gas chromatograph and on a Shimadzu GCMS-QP2010 gas chromatograph/mass spectrometer, both operated with a split/splitless injector and a Shimadzu AOC-20i autoinjector and a Shimadzu AOC-20s autosampler. The column used in both systems was a Supelcowax-10 (polyethylene glycol), 10 m × 0.10 mm I.D. × 0.10 µm film thickness (Supelco, Milan, Italy). Data were acquired by GCsolution/GCMSsolution software.

The high-speed GC analysis of a plasma fatty acid methyl ester (FAME) sample is illustrated in Figure 1. As it can be observed, a complete analysis is achieved in 63 s.

An approximately twice than optimum gas linear velocity (120 cm/s) was applied. This is possible because the employment of reduced ID columns and hydrogen as carrier gas enables the application of higher than ideal velocities with little loss in terms of resolution. Furthermore, a greatly accelerated temperature program (70 °C/min) was used. Fifteen FAMEs were separated and identified through mass spectral probability matching and the interactive use of linear retention indices. The GC run to run time

Figure 1. High-speed GC chromatogram of human plasma FAMEs.



Peak identification: I.S.= $C_{13:0}$; 1 = $C_{14:0}$; 2 = $C_{15:0}$; 3 = $C_{16:0}$; 4 = $C_{16:1\omega7}$; 5 = $C_{17:0}$; 6 = $C_{16:3\omega4}$; 7 = $C_{18:0}$; 8 = $C_{18:1\omega9}$; 9 = $C_{18:2\omega6}$; 10 = $C_{18:3\omega3}$; 11 = $C_{20:3\omega6}$; 12 = $C_{20:4\omega6}$; 13 = $C_{20:5\omega3}$; 14 = $C_{22:5\omega3}$; 15 = $C_{22:6\omega3}$.

(sample introduction, GC analysis and oven cooling) was approximately 3 min. It must be noted that conventional GC applications on this type of sample are generally achieved in a time between 30 and 40 min.

It is well-known that fast GC techniques are characterized by a minimization of analyte band broadening, producing rapid and narrow peaks. Consequently, detector capabilities become very important as rapid elution necessitates fast acquisition rates. Modern FID systems, with sampling rates as high as 250 Hz, are commonly and successfully employed. For example, peak 1 presented a 180 ms width at half height and is one of the most rapid of the entire sample. Accurate integration required a 125 Hz sampling frequency (corresponding to approximately 22 data points for the peak half width) which was 50% of the maximum data acquisition rate.

The future prospects are to entirely automate both the sample preparation and GC analysis through the support of a Shimadzu AOC-5000 autoinjector. The final aim is to perform, simultaneously, FAMEs preparation and the high-speed GC analysis of the formerly prepared samples. A series of major advantages will be attained through method automization: greatly increased sample throughput, excellent analytical repeatability, less risk of contamination and a reduced employment of solvents/reagents.

References

1. E. Matisovà and M. Dömötörovà, *J. Chromatogr. A.*, **1000**, 199 (2003).
2. L. Mondello et al., *J. Chromatogr. A*, **1035**, 237 (2004).

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