Analysis of human plasma fatty

Narrow-bore Column High-speed GC

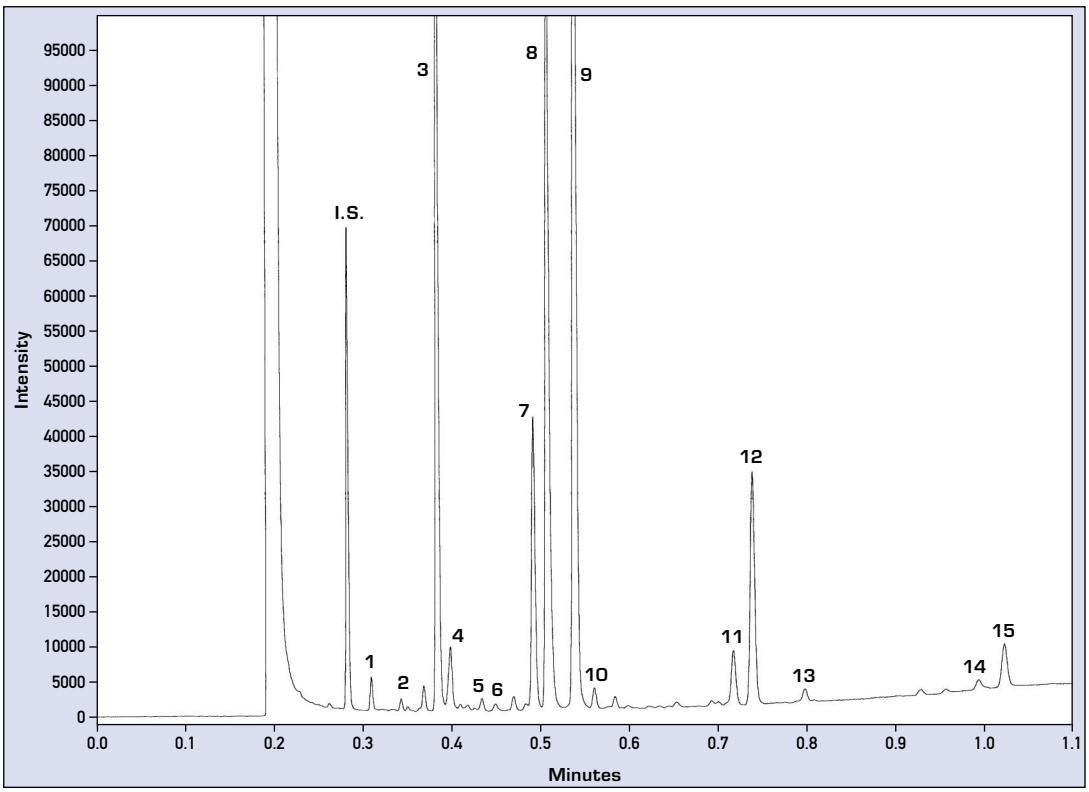

Figure 1. High-speed GC chromatogram of human plasma FAMEs. Peak identification: I.S.: $C_{13:0}$; 1) $C_{14:0}$; 2) $C_{15:0}$; 3) $C_{16:0}$; 4) $C_{16:1\omega7}$; 5) $C_{17:0}$; 6) $C_{16:3\omega4}$; 7) $C_{18:0}$; 8) $C_{18:1\omega9}$; 9) $C_{18:2\omega6}$; 10) $C_{18:3\omega3}$; 11) $C_{20:3\omega6}$; 12) $C_{20:4\omega6}$; 13) $C_{20:5\omega3}$; 14) $C_{22:5\omega3}$; 15) $C_{22:6\omega3}$

Luigi Mondello, Pharmaceutical Faculty, University of Messina, Italy

Present research is based on reduction of analysis times in the separation of human plasma fatty acids (FAs). This is part of a wider research project with the aim of significantly reducing plasma FA analyses times and the cost of clinical assays.

Fast GC maintaining resolution

The major objective of any GC method is the separation of the most critical sample components in the minimum time. This is obviously 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 is 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 sufficient resolving power (compared to traditional GC) for the separation of the compounds of interest. Here, 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 for this delay is merely technical and was due to the lack of suitable GC equipment. Modern GC systems are now capable of supplying the severe experimental conditions that narrow-bore columns necessitate: high inlet pressures, highly controlled split flows, rapid oven temperature heating/cooling and fast electronics for detection.

Sample introduction in Fast GC

The sample introduction system is also critical in this type of analytical approach. Use of a high speed autoinjector is fundamental as it allows the introduction of very narrow sample bands. Furthermore, it also enables extraction of highly reproducible retention time data. Injection band broadening can be further minimized through the use of reduced ID inlet liners (e.g. 0.75 mm).

Columns for Fast GC applications

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

Instrumentation

Analyses were carried out with a Shimadzu GC-2010 gas chromatograph and Shimadzu GCMS-QP2010, both operated with a split/splitless injector and a Shimadzu AOC-20i autoinjector and a Shimadzu AOC-20s autosampler. Column used on both systems was a Supelcowax-10 (10 m x 0.10 mm ID x 0.10 µm film thickness). Data was acquired by GCsolution/GCMSsolution software.

Analysis of human plasma fatty acids with Fast GC

The qualitative/quantitative determination of plasma FAs can be divided basically into two parts: sample preparation and GC analysis. The sample preparation procedure which consists essentially of plasma FA methylic esterification, has been greatly shortened. A thorough description of this initial stage is however outside the scope of this paper.

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

GC detectors for fast analysis of human plasma fatty acids

It is well known that fast GC techniques are characterized by a

acids

minimization of analyte band broadening, producing rapid and narrow peaks. Hence, the signal response remains high as the amount of solute per unit of time that interacts with the detector is not much less than in conventional analysis. 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. A sampling frequency of 50 Hz (commonly employed in 0.1 mm ID column analysis), used in preliminary applications on the plasma sample, proved to be insufficient for precise quantitation of early eluting compounds. 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.

References

1. E. Matisovà, M. Dömötörovà, J. Chromatogr. A. 1000 (2003) 199
2. L. Mondello, A. Casilli, P.Q. Tranchida, R. Costa, B. Chiofalo, P. Dugo, G. Dugo J. Chromatogr. A. 1035 (2004) 237

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