A VERY FAST METHOD FOR THE PREPARATION AND GC ANALYSIS OF HUMAN PLASMA FATTY ACID METHYL ESTERS

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Introduction

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.

Fast GC Maintaining Resolution

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. 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.

Sample Introduction in Fast GC

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 auto injector 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).

Human Plasma Fatty Acids analysis with Fast GC

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

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. The GC run to run time (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.

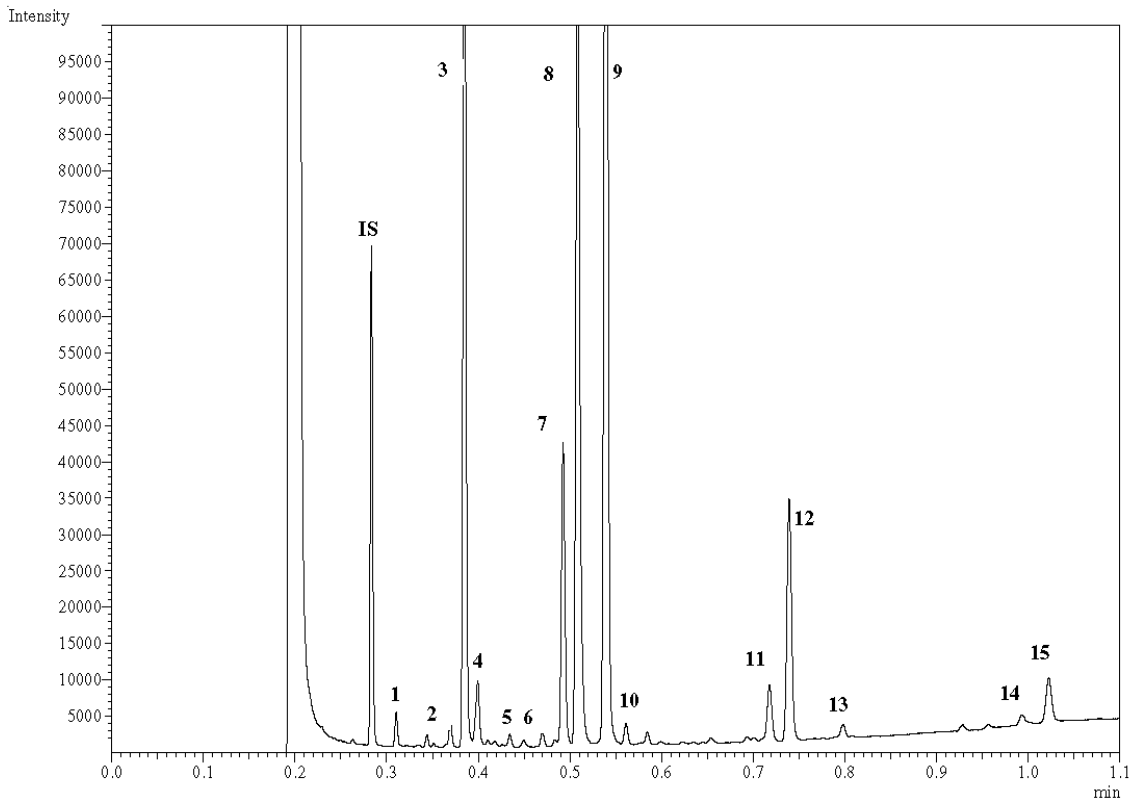

Figure 1: High-speed GC chromatogram of human plasma FAMEs. Peak identification: I.S.: $C_{13:0}$; 1) $C_{14:0}$; 2) $C_{15:0}$; 3) $C_{16:0}$; 4) $C_{16:1\omega7}$; 5) $C_{17:0}$; 6) $C_{16:3\omega4}$; 7) $C_{18:0}$; 8) $C_{18:1\omega9}$; 9) $C_{18:2\omega6}$; 10) $C_{18:3\omega3}$; 11) $C_{20:3\omega6}$;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

Reproducibility

The mean retention times and relative areas (as well as relative standard deviation values) for nine peaks in three consecutive applications are reported in Table 1.

Table 1. Mean retention times, relative areas and relative standard deviation values (RSD) for nine components in three consecutive applications

Peak	Mean ret. time	RSD (%)	Mean rel. area	RSD (%)
1	0.308 min	0.19	0.58 %	2.83
3	0.382 min	0.15	19.56 %	1.35
4	0.396 min	0.15	1.79 %	2.23
5	0.431 min	0.13	0.31 %	2.32
7	0.489 min	0.12	7.11 %	2.60
8	0.504 min	0.11	21.84 %	1.92
9	0.535 min	0.19	34.65 %	1.88
10	0.557 min	0.18	0.58 %	1.40
12	0.734 min	0.16	7.17 %	0.10

As it can be observed from the low retention time RSD values (highest 0.20), there is a very good analytical repeatability. The RSD values (highest 2.83) for the relative areas, although higher, are all within an acceptable range. It can be affirmed that the extreme analytical conditions applied had little or no effect on data repeatability.

Experimental Conditions

Instruments:	GC-2010 with autosampler system AOC-20i/s (Shimadzu)
Column:	Supelcowax-10, 10 m x 0.10 mm I.D. x 0.10 µm film thickness. (Supelco)
Temperature program:	220 °C to 280 °C at 60 °C/min, held for 11 s
Inlet pressure:	567.5 kPa
Inj. temperature:	250 °C
Carrier gas:	H_2, constant linear velocity: 120.1 cm/s
Column flow:	1.53 mL/min
Inj. volume:	0.4 µL; Split ratio: 1:50.
Detector temperature:	300 °C
Sampling rate:	125 Hz; Filter time constant: 10 ms

Data were acquired by GC Solution software

Summary

The present research is based on the reduction of analysis time in the separation of human plasma fatty acids 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. A complete GC analysis is achieved in 63 s. The GC run to run time (sample introduction, GC analysis and oven cooling) is approximately 3 min. It can be affirmed that the extreme analytical conditions applied had little or no effect on data repeatability.

Literature

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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