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A GAS CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF INDIVIDUAL FREE FATTY ACIDS IN SERUM

H. Mohammadiha*

ABSTRACT

Serum fatty acids on 30 healthy Iranian women were determined by a gas chromatographic method.

Oleic and stearic acids were used as standards. The mean values were myristic acid 5.7%, palmitic acid 43%, palmitoleic acid 5.3%, stearic acid 8%, oleic acid 21.3%, and linleic acid 16.4% of total fatty acid serum contents.

INTRODUCTION

The free fatty acid (FFA) fraction of serum has been shown to be composed of variety of saturated and unsaturated fatty acids with chain lengths from 12 to 22 carbon atoms(1). The study of the individual free fatty acids have been handicapped in the past by the inadequacy of the analytical tools available.

With the advance of gas chromatography(2), however, resolution and sensitivity of new orders of magnitude in the microanalysis of fatty acids in achievable, and many new studies have become possible.

A rapid reliable method was needed for the determination of individual fatty acids in blood serum. The method of Wijingarden(3) and Halgren(4), was tried but was found to give erratic results. The method was modified to obtain better extraction efficiency and reproducibility by the use of the diethyl ether in the extraction solvent and by rapid cooling of the reaction mixture after esterfication. Oleic and stearic acids were used as internal standards for the gas chromatographic determination of the fatty acid esters. The results obtained from 30 female controls are given below to show the adequacy of the modified method which could be applied to other blood serum.

* Department of Biochemistry of School of Basic Medical Sciences, University of Teheran, Teheran, Iran.

PRINCIPLE

As in the other chromatographic procedures, analysis by gas chromatography is based on repeated partition of the components of a mixture between a moving phase and a stationary phase. In this case, the moving phase is an inert gas, and stationary one is a thermostable liquid with low vapor pressure selected for its affinity for the substances under analysis.

The usual column used in gas chromatography is ¼ inch diameter glass or metal tube, from 4 to 12 feet long, packed with an inert supporting material of high surface area on which the liquid phase is coated. Inert carrier gas is forced through this column at a controlled rate of 20–100^{cc} per minute.

When a mixture of long chain fatty acids is to be analysed, they must first be converted into esters to make them volatile. The various esters are put on to a column operated at 170°C and have appreciable vapor pressure at this temperature. They are carried through the column at different relative rates. The retention time is the time taken by an individual ester to emerge from the end of the column and it depends on the column packing and the conditions under which it is operated. If these conditions are held constant, the retention time is characteristic of the ester and can be used to identify it, although this time is generally related to the length of the chain and boiling point of the ester, separation of chemically different esters which differ only slightly in boiling point and molecular weight can be accomplished through proper choice of liquid phase.

MATERIAL AND METHOD

5 ml. samples of blood were collected in clean tubes and centrifuged after coagulation was complete. The serum was removed and stored in a refrigerator until analysed a few days later.

The serum lipids were extracted by the modified Folch[5] method as follows:

Two mililiter of the serum were added to 40 ml. of an extraction mixture consisting chloroform-methanol-diethyl ether (2:1:0.1), shaken vigorously and then centrifuged. The proteins coagulated and the linkage of lipoproteins are broken, so all the lipids such as acyle glycerols – phospholipids – free fatty acids – free and estrified cholestrol remain in solution. The organic phase was removed and evaporated to dryness under reduced pressure. The dry residue was dissolved in 5 ml. of methanolic sodium hydroxide and heated under reflux for 5 min. 5 ml. of boron trifluoride in methanol[6] was added

to this solution, which contained the unesterified fatty acids, and the heating continued for a further two minutes.

The mixture was immediately cooled under running water. Saturated sodium chloride solution was added and the heptane phase allowed to settle out. This phase containing the methyl esters of the fatty acids, was removed with a syringe or pasteur pipette and treated with a little anhydrous sodium sulphate under nitrogen to remove traces of the aqueous phase.

One microliter (1 μl.) of this solution is injected into the gas chromatograph.

The analyses were conducted using a Varian 17040/10 series gas-chromatograph fitted with a stainless steel column 5 ft. x ¼ inch diameter, packed with 20% D.E.G.S.(7) on 60/80 mesh chromosorbW–AW. and a flame ionisation detector. Temperatures and flow rates were as follows:

Injector 250°C, column 170°C, detector 290°C, nitrogen carrier gas 17 ml/min., hydrogen 40 ml/min., air 400 ml/min. Reasonable sensitivity was obtained using an attenuation of 128. All the fatty acid ester peaks from the serum were obtained within 25 min. after injection.

RESULTS

The subjects were 30 healthy women aged 18 to 40 years and weight 46–62 kg.

The calculation of the fatty acid contents in serum was based upon comparison of peak heights.

The total FFA content is related to the sum of the areas of all the FFA peaks obtained and the content of an individual fatty acid can be calculated from the ratio of the peak area of the fatty acid to the total peak area of all the fatty acids present. Peak heights can be used instead of peak areas for this calculation with only a small but constant error in the results. This latter technique was adopted in this work and the accuracy checked by adding known amounts of stearic and oleic acids to serum. Satisfactory results were obtained as shown in Table 1 with recoveries of 80 to 85%.

Figure 1 shows a typical gas liquid chromatogram of free fatty acids obtained from blood serum (C14:0– C16:0– C16:1– C18:0– C18:1 and C18:2).

Figures 2 and 3 show a chromatogram obtained from serum to which oleic and stearic acid had been added respectively.

Table 2 shows the actual numerical values obtained from all the samples. The results clearly show the major fatty acid to be palmitic which comprises 43% of the total FFA (corresponding to 258 mg/ml. of serum). The next major acids were found to be oleic (21% corres-

ponding to 128 mg./100 ml. of serum) and linoleic acid (16.4% corresponding to 98.5 mg./100 ml. of serum).

Table 3 shows the statistical analysis of the serum fatty acid contents. Low standard errors were obtained, thus showing the results to be statistically significant.

Table 1
Recovery of fatty acids added to blood serum

Oleic acid added mg/100 ml.	found mg/100 ml.	expected mg/100 ml.	recovered mg/100 ml.	S.D.
–	100	100	100	0
25	119	125	95.2	–4.5
50	140	150	93.8	–6.2
100	187	200	93.5	–6.5
Stearic acid added mg/100 ml.	**found mg/100 ml.**	**expected mg/100 ml.**	**recovered mg/100 ml.**	**S.D.**
–	50	50	100	0
25	72	75	95.5	–4.5
50	94	100	94	–6
100	140	150	93.8	–6.2

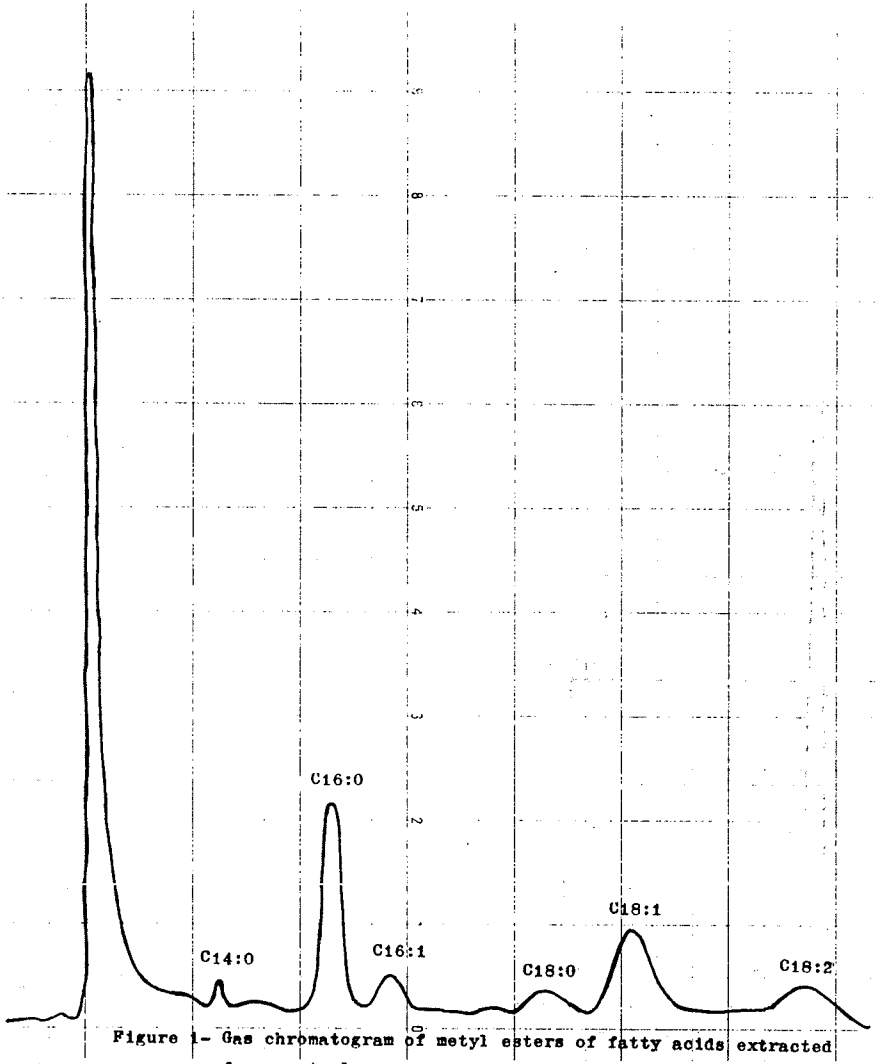

Figure 1- Gas chromatogram of metyl esters of fatty acids extracted from control serum.

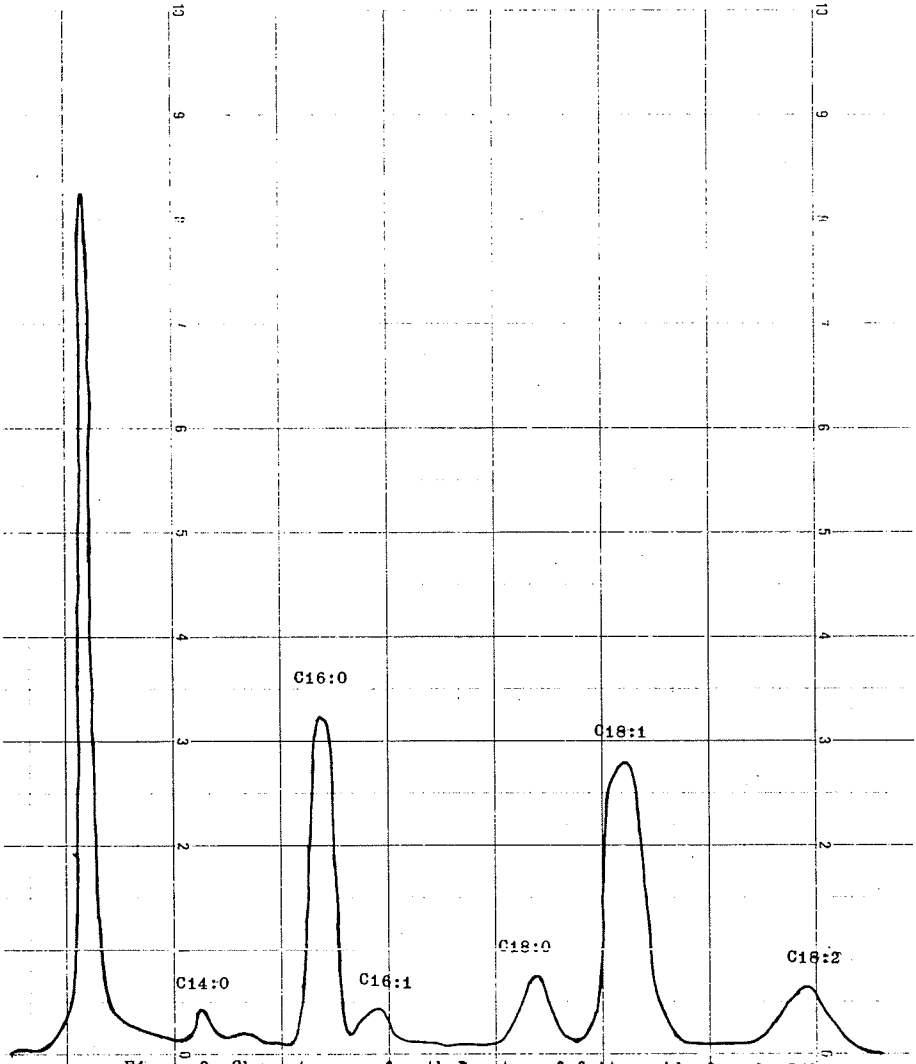

Figure 2- Chromatogram of methyl ester of fatty acids from healthy human blood serum contain 100 mg/100 ml. of added oleic acid.

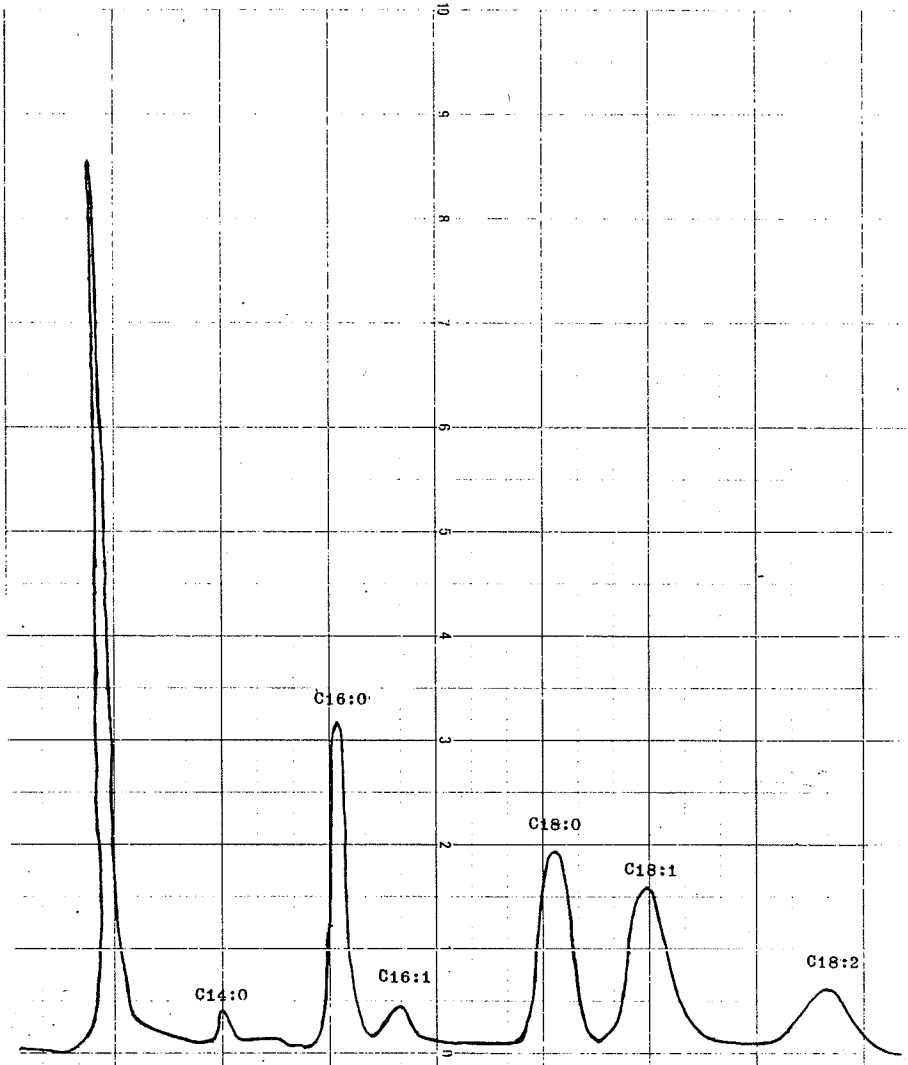

Figure 3- Chromatogram of methyl ester of fatty acids from healthy human blood serum contain 100mg/ ml. of added stearic acid.

Table 2
Fatty acid contents of healthy human blood serum

Fatty acid	Number of tests	Mean of peak height cm.	Percent of total FFA	mg/100 ml. serum
Myristic	30	1.02	5.7	34
Palmitic	30	7.66	43.0	258
Palmitoleic	30	0.95	5.3	30
Stearic	30	1.45	8.2	49
Oleic	30	3.80	21.4	128
Linoleic	30	2.92	16.4	98

Table 3
Statistical analysis of fatty acid contents of healthy human blood serum

Fatty acid	Number of tests	$\bar{X}$	S	S.E.
Myristic C14:0	30	1.02	0.35	0.06
Palmitic C16:0	30	7.66	1.64	0.3
Palmitoleic C16:1	30	0.95	0.24	0.04
Stearic C18:0	30	1.45	0.41	0.07
Oleic C18:1	30	3.80	1.04	0.19
Linoleic C18:2	30	2.92	1.03	0.19

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